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Edited by

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The Invasion of Helminth Eggs by Chytridiacean Fungi.

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Introduction.

The material forming the basis of this paper consists of the two fungi, *Catenaria anguillulæ* Sorokin and *Rhizophidium carpophilum* Zopf, which were found parasitic on the eggs of *Dibothriocephalus latus* in the Helminthology Department in March, 1927. The stool containing the eggs had been washed in laboratory tap water and this was evidently the vehicle of the fungus infection. A sediment obtained from one of the water tanks from which the laboratory is supplied was found to contain infusoria, rotifers and other forms likely to act as hosts for the fungi, and that this was the source of the original infection was proved on more than one occasion by placing some of the sediment in a petri dish along with some uninfected eggs of *D. latus*, when a new infection of both the fungi appeared in the eggs.

In the following paper the fungi are described and an account is given of some experimental work that was carried out with a view to determining the possibility of employing such parasites as a control measure in helminthiasis.

The writers are indebted to Professor R. T. Leiper for suggesting the work and for much helpful advice.

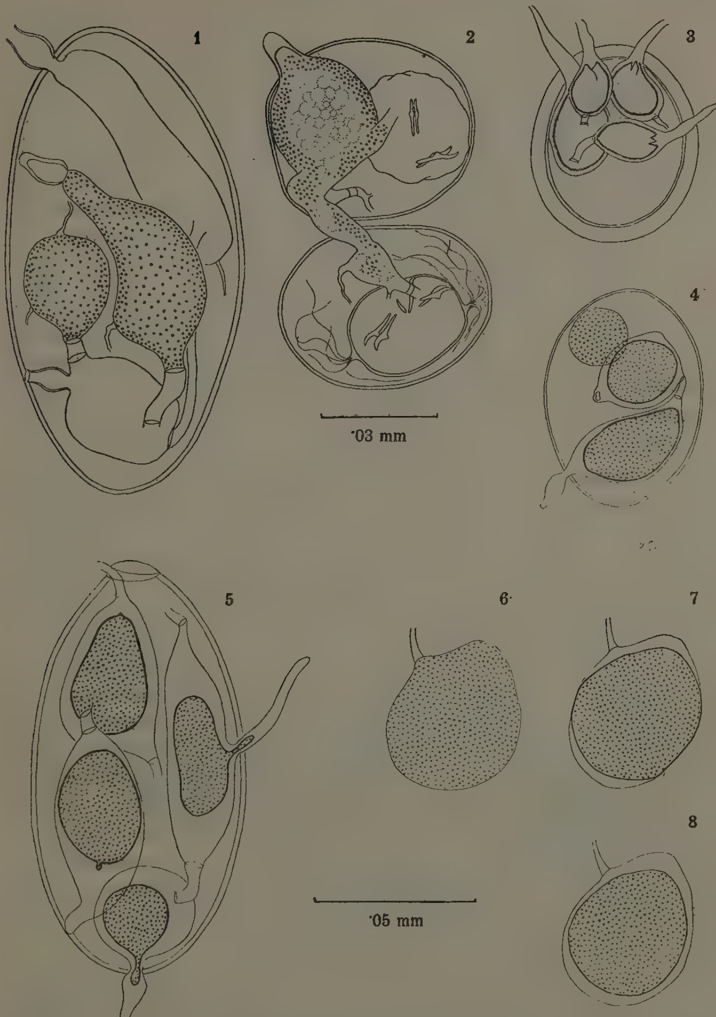
CATENARIA ANGUILLULÆ Sorokin, 1876.

This species was described originally by Sorokin, 1876, who found it growing in eelworms, in encysted infusoria and in the eggs of aquatic "animalculæ." It has since been recorded in various other hosts, notably by Dangeard, 1886, as occurring in *Nitella tenuissima*, by Maupas (fide Seurat, 1920), in several species of free-living nematodes and also in the males of *Hæmonchus contortus*, which had been kept in clear water for several days. The same worker obtained an infection in the larvæ of *Chabertia ovina*. Barlow, 1925, mentions and figures a parasite which is probably *C. anguillulæ* and attention is here drawn to the resemblance between his text figure 12a and figures 5 and 13 of the present paper. Butler and Buckley, 1927, described *C. anguillulæ* in the eggs of *Fasciola hepatica* and Dr. E. J. Butler has recently published a very adequate account on the morphology of this liver-fluke form.

In view of this diversity in the nature of the hosts of *C. anguillulæ*, its occurrence in the eggs of *Dibothriocephalus latus* is not surprising, and other helminth eggs have been experimentally infected. The infection of the eggs of a mite (kindly identified by Miss Susan Finnegan, of the Natural History Museum, as *Rhizoglyphus echinopus*), accidentally introduced in the *D. latus* cultures is worth noting, and on one occasion a dead adult mite was found infected. This seems to be the first record of an arachnoid host for *C. anguillulæ*.

Morphologically this *D. latus* egg form is identical with that described in the eggs of *F. hepatica*, but it differs from the latter in that it has produced structures believed by the present writers to be resting spores. Resting spores have hitherto been unrecorded in this species, but it is probable that in Barlow's fig. 12a, is represented a structure identical with those here described.

Fig. 1.—An egg of *Fasciola hepatica* with a typical infection. Showing dichotomy of the thallus. Fig. 2.—Two eggs of *Hymenolepis diminuta* infected. An unusual case in which the mycelium has penetrated the thin shell and attacked another adjacent egg. Fig. 3.—Four dehisced resting spores in an egg of *Parascaris equorum*. Fig. 4.—Egg of *D. latus* with two connected resting spores and one solitary sporangium. Fig. 5.—*Fasciola* egg with four resting spores. In two of the resting spores retraction from the dehiscence tube is not complete. Figs. 6, 7 and 8. Showing contraction of the contents of a sporangium to form a resting spore. Interval of three hours between Figs. 6 and 8. (The 0·03 mm. scale refers *only* to Fig. 2).



CATENARIA ANGUILLULÆ.

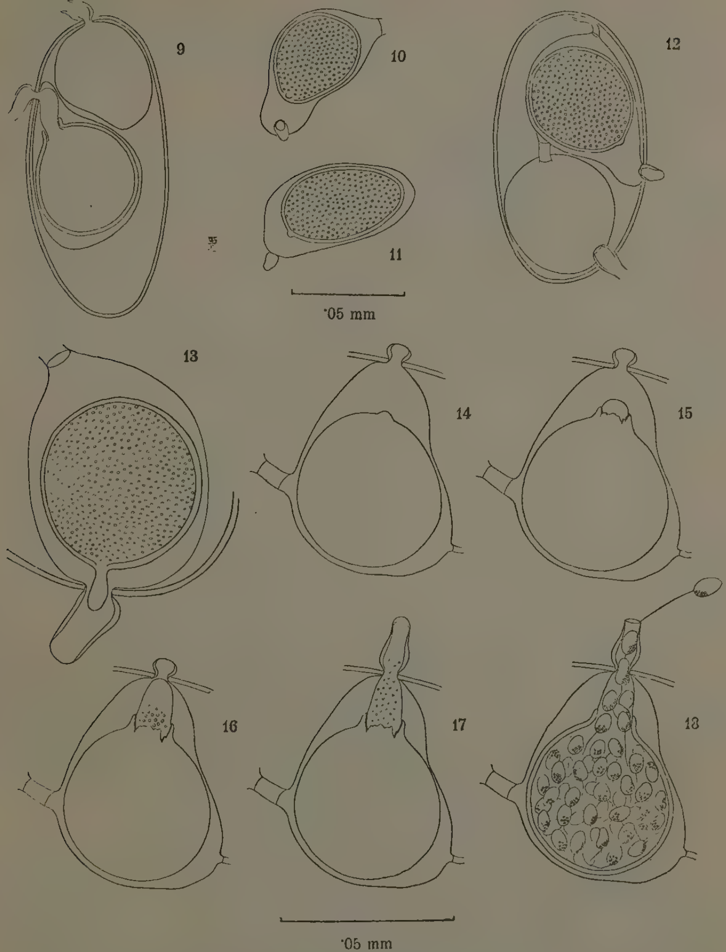
Figs. 1 to 8.

The Resting Spores of Catenaria anguillulæ.

It may possibly not be considered out of place here to give a brief outline of the life cycle of *C. anguillulæ* occurring in a helminth egg. A sporangium or spore-sac having grown almost to maturity at the expense of the egg contents, bores a hole through the egg-shell and sends out a tube to the exterior. Through this a large number of active uniciliate zoospores, measuring about $4 \cdot 5\mu$ by $7 \cdot 5\mu$ escape from the sporangium and after swimming about for a varying period infect other eggs. This takes place as follows : a zoospore settles down on the surface of an egg, loses its cilium and forms a membrane about itself. It bores a very minute hole through the shell and passes in leaving the empty membrane outside. Inside the egg it appears as a rounded mass which sends out a long mycelium through the egg substance. This thickens, becoming obviously tubular and later swells at intervals along its length. The swellings become the sporangia, and are separated from one another by a length of the tubular mycelium which become septate at its unions with the sporangia. An absorptive system consisting of a few very delicate rhizoids may sometimes be seen arising from the sporangia or from the mycelium.

Amongst the *D. latus* eggs infected with *Catenaria*, some were observed to contain sporangia which possessed a double wall, the inner one usually being considerably thicker than the outer one. It was thought at first that this might be a new species of *Catenaria*, and in order to obtain a pure culture of it an egg containing only this type of sporangium was isolated, washed in boiled water and added to some fresh eggs which had been boiled to exclude any possibility of an outside infection. For convenience of observation the larger eggs of *Fasciola hepatica* were used in this culture. In the infection that subsequently appeared, both types of sporangium were formed, and this result followed a similar experiment in which the isolated egg contained only the single-walled form. The conclusion drawn from these results, that only a single species was involved, was later confirmed when single and double walled sporangia were found interconnected with mycelium (fig. 12).

Fig. 9.—*Fasciola* egg with one dehisced resting spore and one dehisced sporangium. Figs. 10 and 11.—Typical resting spores. Fig. 12.—Resting spore and sporangium with connecting mycelium. Fig. 13.—Resting spore showing incomplete retraction from the dehiscence tube. Figs. 14 to 18.—Dehiscence of resting spore. (Fig. 15 drawn at 3.15 p.m., Fig. 16 at 3.40 p.m., Fig. 17 at 4.30 p.m., Fig. 18 at 7.30 p.m.).



CATENARIA ANGUILLULÆ.

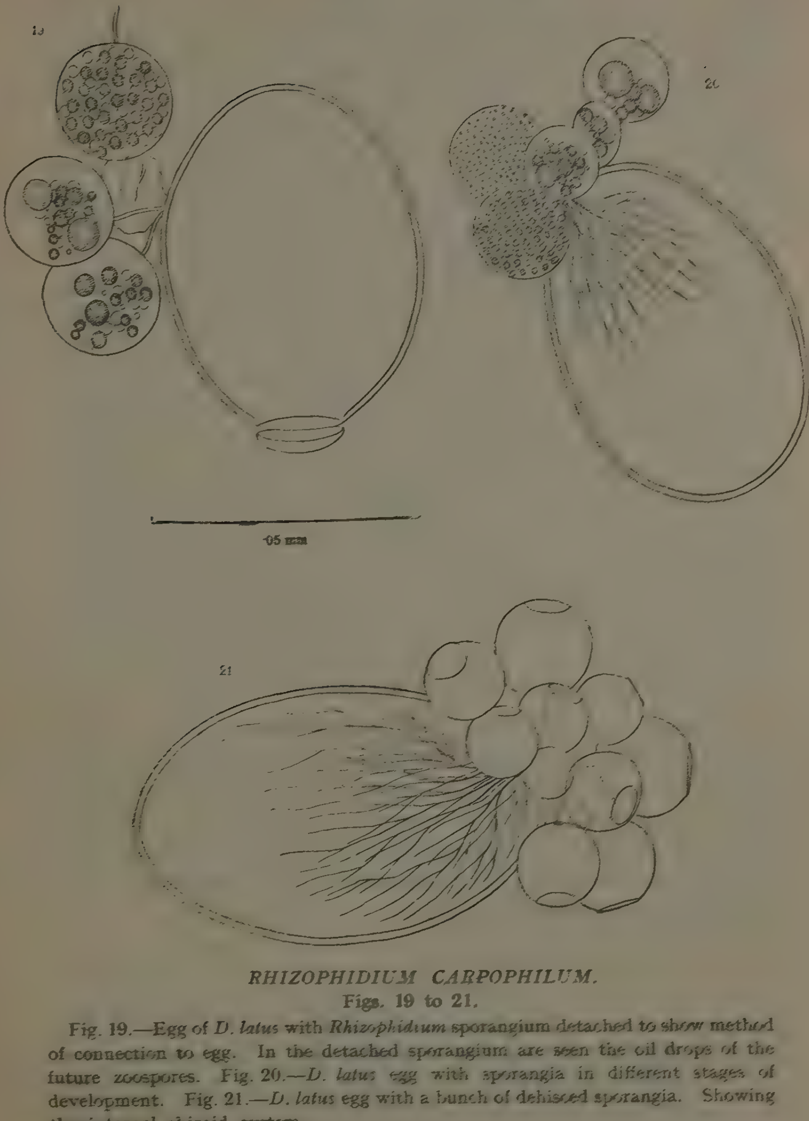
Figs. 9 to 18.

That these double-walled sporangia were in fact resting spores, was concluded from the relatively thick inner wall, their power of surviving desiccation, and from the fact that they could remain quiescent for varying periods up to two months. (The maximum period has not been determined.)

The formation of a resting spore takes place simply by the contraction of the finely granular contents of an ordinary sporangium to form a roundish mass surrounded by a membrane, which is later replaced by the thick wall (figs. 6 to 8). In shape the resting spores vary from spherical to irregularly oval, and generally bear a resemblance to the sporangium from which they are derived. In size there is a rough proportion between resting spores and sporangia. The smallest resting spore seen was 12μ in diameter and the largest was 65μ by 50μ. In the majority of cases they were observed to have been formed subsequent to the formation of a dehiscence tube by the sporangium. The contents of the tube in such cases also undergo contraction and are drawn back entirely from it (with occasional exceptions, as in fig. 5) and are represented in the resting spores by a very characteristic prominence or " pip " on its surface nearest to the empty tube. The " pip " indicates the point at which the resting spore bursts preparatory to the liberation of zoospores. This bursting results in an aperture with jagged edges through which a membrane protrudes, enlarges and finally forms a wide tubular connection between the resting spore and the aperture in the egg shell previously made by the sporangium (figs. 14 to 18). In the instance figured the membrane proceeded through this aperture and formed an external dehiscence tube in place of the old one which seemed either to have deteriorated or was never completely formed.

RHIZOPHIDIUM CARPOPHILUM Zopf, 1884.

The parasite of the eggs of *D. latus* is assigned to the above species with some reserve, for it exhibits certain differences in form and habit that may necessitate the formation of a new species. On the other hand, the possibility that these differences may be a reaction on the part of the parasite to the abnormal host, must not be disregarded; and unless they remain constant in the *D. latus* species, after it has been cultured upon the host on which Zopf's species was found, it seems undesirable to distinguish it specifically from *R. carpophilum*.



RHIZOPHIDIUM CARPOPHILUM.
Figs. 19 to 21.

Fig. 19.—Egg of *D. latus* with *Rhizophidium* sporangium detached to show method of connection to egg. In the detached sporangium are seen the oil drops of the future zoospores. Fig. 20.—*D. latus* egg with sporangia in different stages of development. Fig. 21.—*D. latus* egg with a bunch of dehisced sporangia. Showing the internal rhizoid system.

The sporangia of the *D. latus* form are perfectly spherical and vary in diameter from about 10μ to 35μ. The single, circular dehiscence aperture varies in diameter from about one-third to two-thirds that of the sporangium. These sporangia show a tendency to occur bunched together on an egg in a group containing up to about thirty. In such a group (fig. 21) many of the sporangia are not in contact with the egg, and their relation to it is not obvious unless they are detached with a needle, when the method of connection is seen to be by a single delicate stalk. Singly occurring sporangia rarely possess this connecting stalk, the sporangium itself usually being in contact with the egg, and it would appear that the presence of the stalk is correlated with the bunching habit. Inside the egg a very delicate, profusely branching system of rhizoids can be seen, but only under a high magnification, arising from the inner surface of the egg shell at the point of contact of the sporangium. In the case of a bunch of sporangia, the points of origin are numerous but arise within a limited focus as in fig. 21. The zoospores are spherical with a diameter of $4 \cdot 5\mu$ to $5 \cdot 5\mu$ and have a spherical excentric oil drop of about 2μ diameter, and a long posterior cilium. They emerge at first very slowly from the sporangium in a mass which after five or ten minutes breaks up and the zoospores swim away. The remaining spores then swarm actively and emerge one by one. The empty sporangium is seen to be composed of a very delicate transparent membrane which often collapses and loses its spherical shape.

The main points of difference between *Rhizophidium carpophilum*, Zopf and the present form seem to lie in the dimensions of the sporangia, Zopf's form rarely exceeds 20μ in diameter, in the sparsely branched rhizoids of the former and in the continually sessile habit of its sporangia. The one exception to this latter point is to be seen in Zopf's, fig. 15, Pl. IX in which one of the sporangia is distinctly separated from the surface of the oogonium by a short length of rhizoid.

Experiments.

In areas of endemic helminthiasis where the method of sewage disposal results in the precipitation of the contained eggs in large bulk and their destruction by chemical means is not practicable, for example in septic tanks, this result might possibly be achieved by the introduction of fungus

parasites. That such parasites do sometimes act naturally as a control is attested to by Barlow, 1925, working on the life cycle of *Fasciolopsis buski* in Shaoshing, who states "Freezing kills many eggs, but the greatest number is killed by natural enemies." As an example of this he figures the egg parasite referred to earlier in this paper.

That both *Catenaria* and *Rhizophidium* are efficient in ordinary laboratory conditions in destroying large numbers of eggs in a comparatively short time is demonstrated by some experiments described under the heading "Rate of Infection." Having in view the septic tank environment, experiments were set up to find the viability of the fungi in anærobic cultures. Attempts to culture the fungi in putrefactive and semi-fluid conditions were disappointing and it would appear that both forms flourish best in comparatively fresh conditions.

The large range of hosts of *C. anguillulæ* has already been commented upon and relatively thin-shelled helminth eggs such as those of *D. latus D. mansoni* and *Fasciola hepatica* are readily infected. Infections were also obtained in the eggs of *Parascaris equorum*, but most successfully in those eggs which were infected shortly after being taken from the uterus. Those having morulæ developed were rarely attacked and fully embryonated eggs were immune. While similar results were obtained with *Rhizophidium* it is improbable that this form, owing to its delicate nature and unprotected external sporangia could be of practical use.

ate of Infection.

It has previously been found by experiment that *Catenaria* will not complete its life cycle at laboratory temperature in less than four days. While this result holds good for laboratory conditions, yet the time necessary for the completion of the life cycle is less under certain conditions, *e.g.*, at higher temperatures. Another condition producing a rapid life cycle was when the fungus was cultured in the eggs of a round worm—*Parascaris equorum*. Free zoospores of *Catenaria* were placed in a watch glass containing the nematode eggs on January 31st, 1929, and empty sporangia within the eggs were observed, February 2nd, 1929. The total life cycle had occurred within two days. In order to discover the effect of external conditions on the rate of infection, cultures were allowed to develop under known conditions and accurate counts made at known intervals of time. The method used was as follows :—a shallow

cell, 3 cms. square was used, the base of which had previously been marked out into small squares with an area of 4 square mm. When the culture had been proceeding for the required length of time some of the eggs were transferred to this cell and spread fairly evenly. The cell was then sealed by means of a glass cover and the ova counted under a two-inch objective. A second count with a two-third inch objective was then made of the ova infected with the fungus and the percentage of infected ova calculated. The number of ova counted from each culture usually varied between 3,000 and 4,000.

The following results were obtained for *Catenaria* :—*Culture A* containing uninfected *F. hepatica* ova and distilled water was put in a autoclave for sterilisation and five sporangia of *Catenaria* in *D. latus* were added. Five days later 17·9 per cent. were infected and after three more days 21·6 per cent. were infected.

Culture B was similarly treated but tap water replaced the distilled water. After five days 55 per cent. were infected and after three more days 62·1 per cent.

Culture C contained an infected *D. latus* ovum, uninfected *Fasciola* ova and septic water containing many protozoa and bacteria. After five days 10 per cent. of the ova and after three more days 16·8 per cent. were infected.

It is obvious from these results that while *Catenaria* will live and reproduce in water containing septic substances, these substances inhibit its development to a marked degree.

Another culture was examined every few days and development noted :—

Three days after sporing of the infected egg the first generation was seen.

After four days 6 per cent. After eight days 7·5 per cent. After eleven days 28·5 per cent. After thirteen days 34 per cent. After nineteen days 36 per cent. were infected. Thereafter the percentage of infected eggs grew gradually larger but the culture finally died out though 12 per cent. of the eggs remained uninfected.

A culture of *Rhizophidium* on *D. latus* eggs was similarly counted. Here the life cycle is much quicker. Two days after sporing 10 per cent. of the ova were infected and two days later 65 per cent. had been attacked.

Anærobic Conditions.

Two experiments were set up to test the viability of sporangia under anærobic conditions. In the first experiment one ovum containing nearly mature sporangia together with some uninfected ova was placed in each of four watch glasses and the vessels filled up with filtered sewage having a high oxygen requirement. Each was then sealed with a glass cover coated with vaseline, care being taken to avoid enclosing any air bubbles.

Watch glass *A* was provided with pure sewage.

Watch glass *B* was provided with sewage and water in the proportions 3 : 1.

Watch glass *C* was provided with sewage and water in the proportions 1 : 1.

Watch glass *D* was provided with sewage and water in the proportions 1 : 3.

A control *E* was provided with distilled water.

In *B*, *C* and *D* some of the sporangia discharged their spores but no further infections occurred: in *A* dehiscence tubes were formed but the contents of the sporangia contracted away from the wall and died. Infection of uninfected eggs occurred only in *E*, the control. These results suggest that oxygen is not essential for the full development of *Catenaria* and it was thought possible that the sewage contains some substances toxic to the zoospores. Therefore a further experiment was carried out. Four test-tubes *A* to *D* were half-filled with water, some uninfected ova were added and liquid paraffin was poured in to a depth of half-an-inch. The contents were then boiled vigorously for several minutes to expel all dissolved oxygen: the paraffin preventing re-absorption during cooling. Four more test-tubes *E* to *H*. were treated in a similar manner except that a piece of animal charcoal was introduced before boiling: this would absorb any oxygen which might be introduced with the infected ova: four other test-tubes, *I* to *L*, were similarly treated and immediately after the introduction of the infected ovum they were further sealed with a layer of paraffin wax. Infected ova were added to each by means of a very fine pipette, care being taken to add a minimal amount of water.

To *A*, *E* and *I* was added *Catenaria* and the culture kept at 37° C.

To *B*, *F* and *J* was added *Catenaria* and the culture kept at laboratory temperature.

To C, G and K was added *Rhizophidium*, and the culture kept at 37°C.

To D, H and L was added *Rhizophidium* and the culture was kept at laboratory temperature.

These cultures were set aside for a week when some of the eggs were extracted with a fine pipette. It was then found that in the cultures of *Catenaria* which had been kept at 37° C., *i.e.*, A, E and I, development had occurred and a large number of the eggs had been infected. Both types of sporangia were present but the zoosporangium predominated. No other infections were noticed until the cultures were examined four days later—that is eleven days after the experiment had been set up. A few infected ova were then observed in the remaining *Catenaria* cultures, that is in B, F and J. No infection of *Rhizophidium* occurred throughout the experiment. These results show that *Catenaria* can complete its full development under anærobic or nearly anærobic conditions and that the rate of development is not appreciably slowed down.

Semi-fluid and Putrefactive Conditions.

To observe the behaviour of *Catenaria* in the above conditions a test-tube was half filled with some washed stool containing a large proportion of *D. latus* eggs and some infected *F. hepatica* eggs were added to the supernatant fluid after the material had settled down. Six weeks later a layer of this material to a depth of about ⅛-inch was removed with a wide-bore pipette and the contained *D. latus* eggs were examined to see if the infection had penetrated the semi-fluid mass. No infected eggs were found.

A similar experiment was done using about 1 cc. of *D. latus* material in a small test-tube. About twenty *Catenaria* infected *Fasciola* eggs were added and the mass shaken up. After a control, consisting of the same material diluted in a large quantity of tap water, had shown an infection of about 50 per cent. of *D. latus* eggs, the material in the test-tube was examined but only a single *D. latus* egg was found infected. Entirely negative results followed a similar experiment in which *Rhizophidium* was used.

Desiccation.

A large number of ova of *D. latus* and *F. hepatica* containing sporangia of *Catenaria* were isolated in solid watch glasses and the supernatant

fluid allowed to evaporate slowly. After being left dry for known periods varying from one minute to eight days distilled water was added and development allowed to continue. The watch glasses were sealed by a glass cover smeared with vaseline. It was found that no sporangium would spore if it had been dried for more than 120 hours, the protoplasm having plasmolysed and coagulated. It is worth noting that a short period of dessication—up to about twenty-four hours—acts as a stimulus to early sporing ; for instance, a *D. latus* infected ovum which was dried for ten hours on November 5th spored the next day : a control set aside at the same time did not spore until November 15th.

In order to discover the percentage of sporangia which will survive desiccation, ten well infected *F. hepatica* eggs were dried in each of eleven watch glasses for various periods up to 140 hours after which they were wetted. The following results were obtained :—

A	dried	12	hours	—41	sporangia	spored =	91	per cent.
B	,,	24	,,	36	,,	,,	73	,,
C	,,	36	,,	34	,,	,,	68	,,
D	,,	48	,,	17	,,	,,	35	,,
E	,,	61	,,	11	,,	,,	20	,,
F	,,	71	,,	8	,,	,,	18	,,
G	,,	84	,,	6	,,	,,	11	,,
H	,,	96	,,	5	,,	,,	9	,,
I	,,	110	,,	3	,,	,,	6	,,
J	,,	120	,,	2	,,	,,	4	,,
K	,,	140	,,	0	,,	,,	0	,,

Temperature.

A large number of uninfected *F. hepatica* ova together with one infected ovum were placed in an incubator kept at a temperature of from 14° C. to 18° C. When a fair number of ova were infected the culture was divided into three portions.

A acting as control remained in the incubator.

B was placed in an incubator kept constant at 37° C.

C was placed in an ice-chest the temperature of which varied between 4° C. to 8° C., but which remained on the whole about 5° C.

After a week it was found that the sporangia in *C* had been unable to withstand the low temperature and were all dead. Culture *A* showed a fair percentage of infected ova the sporangia being almost entirely

zoosporangia : culture *B* had become well infected only about 25 per cent. of the ova remaining free from infection : about 30 per cent. of the sporangia had double walls. This percentage did not increase with further infection, later formed sporangia being mainly of the non-resting type ; similarly a culture which had developed entirely at a temperature of 37° C., showed only zoosporangia. This result suggests that a sudden rise of temperature may be instrumental in causing the production of resting sporangia but as this was a single experiment the result can only be taken as suggestive. Development proceeded much more quickly at a temperature of 37° C. : the condition seemed to be favourable to the fungus. It was noticed that a fair percentage of embryonated ova were attacked in this culture.

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Plate 1 (×370).

Fig. 1.—*D. latus* egg infected with *Rhizophidium*. Note the numerous sporangia, some of which are " stalked."

Fig. 2.—*D. latus* egg with two sessile sporangia of *Rhizophidium*.

Fig. 3.—*D. latus* egg with *Rhizophidium*. Note the two empty sporangia.

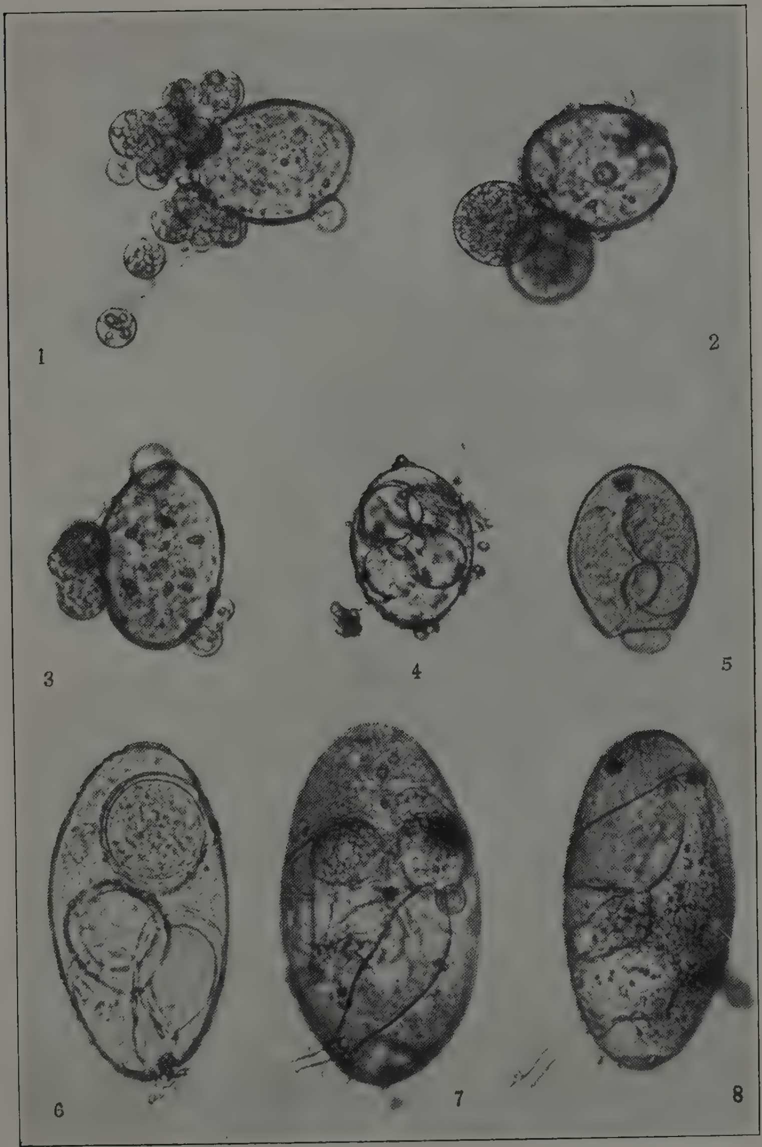
Fig. 4.—*D. latus* egg infected with *Catenaria*. Empty resting spores.

Fig. 5.—*D. latus* egg with living resting spores of *Catenaria*. Double walls not apparent.

Fig. 6.—*F. hepatica* egg with *Catenaria*. Three resting spores two of which are empty and one empty sporangium. Note double walls of resting spores.

Fig. 7.—*F. hepatica* egg showing two resting spores and one empty sporangium with dehiscence tube.

Fig. 8.—*F. hepatica* egg with two empty sporangia with dehicence tubes.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Journal of Helminthology, Vol. VII, *No.* 1, *March*, 1929, *pp.* 15-26.

On the larval migration of some parasitic nematodes in the body of the host and its biological significance.[1]

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As Looss has shown, the larvæ of *Ancylostoma* can infect a suitable host by penetrating the healthy skin. If we place the infective material on the surface of the skin, after a short time we find the larvæ in the subcutaneous tissue. The same behaviour is shown by the filariform larvæ of *Strongyloides stercoralis*, which provide more convenient material for examination, because they can be collected very easily in pure culture and free from fluid by the special culture-method described by me (1924), the principle of which depends on the well-known fact that the filariform larvæ of *Strongyloides* and other nematode larvæ, accumulate in the form of white filaments of several millimetres in length (fig. 3), each consisting of thousands of individual larvæ, on little prominences in their vicinity, all being guided to similar places by the same sets of "tropisms" (Fülleborn, 1924).

Another form of tropism of the *Strongyloides* larvæ, and also of the larvæ of *Ancylostoma* and other nematodes, is *thermotaxis* as discovered by Khalil. If we place a "filament" composed of *Strongyloides* larvæ as described above on the surface of an agar plate and put in the vicinity of them a piece of heated metal all the larvæ begin to march in the direction of the source of the heat like a regiment of soldiers (fig. 1). Thermotaxis will very effectively assist the larvæ parasitic for warm-blooded hosts to penetrate the skin, but, according to the experiments of Goodey (1924) and myself (1928), the larvæ of *Rhabdias*

[1] A lecture delivered, under the auspices of the University of London, at the London School of Hygiene and Tropical Medicine, on 11th March, 1929.

fuscovenosa and *Rhabdias bufonis* of the cold-blooded snake and frog (in which thermotaxis cannot facilitate the entrance of the larvæ into the skin) also show thermotaxis, a fact suggesting that it may be developed in some nematode larvæ independent of *parasitic* habits. On the other hand, according to the experiments of Kosuge, made in my laboratory, *Strongyloides* larvæ can penetrate the skin without the help of thermotaxis, entering the cold skin of a *dead* mouse and also the skin of a frog, and under favourable conditions, they can even enter a *Daphnia* (Fülleborn, 1914) or inorganic material ; the act of entering a body being apparently a purely mechanical phenomenon, and not influenced by any specific " histotropism."

According to Looss, after entering the tissue of the skin the hookworm larvæ carried by the circulation of the blood and the lymphatic vessels reach the right heart. Then, with the blood of the right heart the larvæ reach the lungs but are arrested in the thin capillaries of the air-vesicles. By perforating the delicate walls of the capillaries and of the air-vesicles, they reach the bronchioles, whence, ascending the bronchi and the trachea, they come to the pharynx, are swallowed with the saliva, and finally arrive in the intestine, where they develop to sexual maturity.

Sambon was quite right in his objection that, for biological reasons, it seemed difficult to assume in the larvæ such a wonderful instinct, leading them from the lungs through the trachea and the pharynx to their destination in the bowels, and he suggested that the great majority of larvæ are carried from the lung to the intestine with the circulation of the blood or the lymph.

By making a section either across the whole trachea or across the œsophagus of a dog, and then infecting its skin with the larvæ of *Ancylostoma* and *Strongyloides*, I have tried to decide the question experimentally in collaboration with Schilling-Torgau. Some time after the skin infection, many *Strongyloides* larvæ as well as *Ancylostoma* larvæ were found in the mucus, collected in the canula inserted in the lower end of the trachea. After cutting, not the trachea but the œsophagus, the larvæ were evacuated with the saliva.

Indeed, there was no doubt that the great majority of the larvæ were following the route indicated by Looss. They are not led, however, by a " wonderful instinct," but, as I could prove experi-

mentally, transported merely mechanically by the action of the ciliated epithelium of the air passages up the bronchi and trachea to the pharynx (Fülleborn, 1925).

However, although the passage from the lungs to the stomach *via* the trachea and the œsophagus was broken completely by our operations, in these experiments some few larvæ always reached the intestine ; these were evidently larvæ that had previously reached the left heart and the systemic arteries by passing, in the lungs, from the pulmonary arteries to the pulmonary veins, as is discussed below.

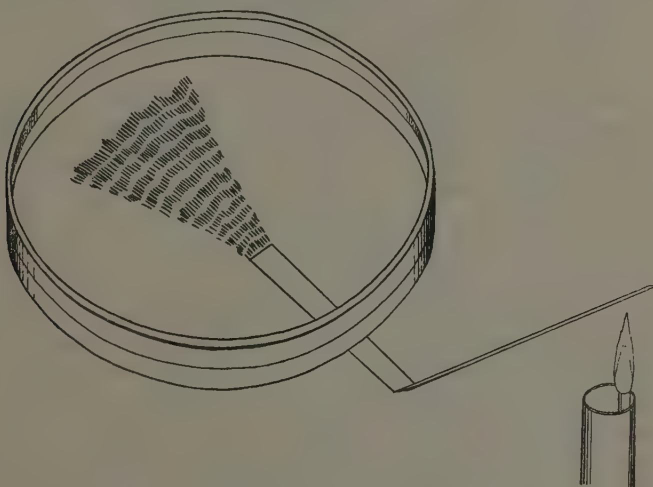

Fig. 1.—Filariform strongyloides larvæ migrating on an agar-plate under the thermotactic attraction of heated metal placed underneath the petri-dish.

The larvæ thus transported with the blood of the aorta, reach not only the intestine (where they develop to sexual maturity) but all the organs of the body. If the presence of nematode larvæ in the systemic circulation is suspected, it is advisable to inspect with a hand lens the surface of the kidney (fig. 4). There the larvæ produce very characteristic small hæmorrhages in the convoluted tubules by filling some loops with the thin stream of blood which follows the individual larva, when it bores out from a ruptured blood vessel into the adjacent renal tissue (Fülleborn & Schilling-Torgau, 1911 ; Fülleborn, 1914 and 1920). This happens

not only in the loops of the glomeruli, as described for *Ascaris* larvæ by Yokogawa, but also in the capillaries of the cortex, and, as shown by Yamaguchi in my laboratory, especially in the uppermost layers of the organ.

The reason for this unequal distribution of the larvæ in the kidney and also in other organs seems to be, that the larvæ, being heavier than the blood, are conveyed only in the central and not in the peripheral part of the blood-stream. Thus in blood, containing larvæ, streaming in an apparatus, as shown in fig. 2, the vast majority follow the straight and not the lateral branch of the apparatus; and the same happens if the experiment is conducted not with glass and india-rubber tubes but with the arteries of an animal.

The reason why so many of the larvæ streaming in the interlobular arteries of the kidney reach especially the capillaries of the uppermost surface of the organ, seems to be that in consequence of the peculiar type of ramification of this artery these capillaries receive, perhaps, relatively, more from the blood of the central stream of the vessel than do its lateral branches supplying the glomeruli (Fülleborn, 1925, pp. 18-38).

On the other hand experimental injections of larvæ into arteries show that the frequency and extent of bleeding depend very much on the structure of the tissue invaded by the boring larvæ. For example, in the brain hæmorrhages take place only in the loose tissue of the meninges, practically never in the nervous substance, and therefore even quite massive injections of larvæ are usually tolerated by the brain without any perceivable damage to the animal; a fact not without some clinical interest (Yokogawa, Yamaguchi, Fülleborn, 1925). However, after heavy experimental infections with the larvæ of *Ascaris lumbricoides*, according to Suyemori, bleeding in the retina can occur.

It is true that not all the larvæ injected *via* the carotid artery into the brain arteries, will break out of the blood vessels, since the systemic capillaries—according to my experiments, which I cannot discuss here in detail (Fülleborn, 1925 and 1926 b.)—permit the passage, not only of living larvæ of *Strongyloides*, but also of larvæ that were killed before injection, and even of bodies from about 20μ in diameter (*e.g.*, coccidial cysts) injected into the arteries.

On the other hand the very narrow capillaries of the lung always entirely retained such dead material: and if living *Strongyloides* and

Ancylostoma larvæ are captured in them, their violent movements must break the delicate walls separating the air vesicles. The fact that, nevertheless, of the living larvæ injected *via* the jugular vein into the right heart, some few very soon reach the systemic arteries indicates, I think, that these larvæ have succeeded in boring from the inner surface of the air passages into the lumina of pulmonary veins. At any rate, so far as the entrance of living *Strongyloides* larvæ into the systemic circulation is concerned, it made (even quantitatively) no difference whether

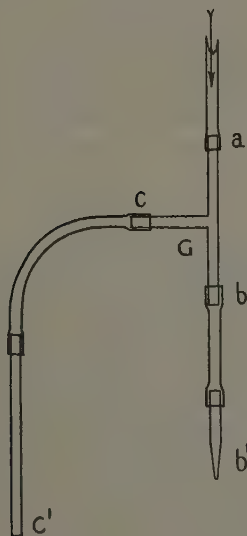

Fig. 2.—System of tubes to demonstrate the tendency of larvæ to be transported only in the "central stream."

the material was injected into the pulmonary artery or through the trachea directly into the air passages.

This holds good, I believe, not only for the larvæ of *Strongyloides* and *Ancylostoma*—with a diameter of about 15μ and a little over 20μ respectively—but also for the great majority of the larvæ of *Ascaris lumbricoides*, which are only 12-13μ thick.

For injection experiments the *Ascaris* larvæ could be collected in great quantities by placing the chopped up liver or lung of a guinea-pig, recently injected with matured *Ascaris* eggs, on a layer of gauze instead of a dialysing membrane spread over the bottom of the funnel-shaped interior of an ordinary glass dialyser. In the incubator at body temperature nearly all the larvæ bore out of the tissue, fall through the meshes of the gauze and can be collected from the bottom of the outer vessel of the dialyser (Fülleborn, 1925).

This method, used by us for many years in the detection of nematode larvæ in tissue, is practically the same as that recommended lately by Allen Smith, who puts the cut organs in a " Baermann funnel " ; the principles of the method were first described by Yoshida (1916).

The scanty tissue-detritus that has passed with the larvæ through the meshes of the gauze can easily—and without damage to the larvæ—be removed by digesting it with the freshly discharged juice from the duodenal fistula of a dog, and the material is then ready for injection into the blood vessels.

If, instead of placing the *Strongyloides* larvæ from the culture on the skin of a dog, we introduce them directly into his stomach, according to my experiments in collaboration with Schilling-Torgau the great majority, deprived of oxygen, suffocate there in a short time, with the exception of a few individuals that succeed in boring into the tissue of the stomach walls in the same manner as when they are placed on the surface of the skin. With the circulation these larvæ reach first the liver, then the right heart and the lungs, and return *via* the trachea to the stomach. But such larvæ are no longer liable to be suffocated in the stomach, and in consequence of biological and morphological changes that have taken place during their stay in the tissue, they are able to develop to sexual maturity in the intestine of their host.

According to recent experiments in collaboration with Dr. Lee this holds good not only for the " indirect " filariform larvæ of *Strongyloides* descended from the free-living sexual generations of the parasite, that were used in my former experiments with Schilling-Torgau, but apparently also for the filariform larvæ that develop " directly " from the rhabditiform larvæ evacuated with the fæces. The assertion of Nishigori that such " indirect " filariform larvæ of *Strongyloides* can become sexually mature without leaving the body of the host has so far not been confirmed by our experiments.

As is well-known, in 1913 Miyagawa, using in his experiments the operative methods introduced by Schilling-Torgau and myself but feeding to his dogs the larvæ of *Ancylostoma caninum* instead of those of *Strongyloides*, came to the conclusion that the former when swallowed were also obliged to migrate *via* liver and lung back to the stomach, before they could mature in the intestine. Here I wish to point out with emphasis, that it is Miyagawa and not myself—as so often cited incorrectly —who is responsible for these statements so far as *Ancylostoma* is involved. These statements of Miyagawa, although generally accepted, were hardly correct, as shown in 1925 by Yokogawa & Oiso ; and according to my recent experiments (Fülleborn 1926A and 1927) the larvæ of *Uncinaria stenocephala* the " Necator " of the dog, can also develop in the intestine without previous migration. One of my experiments was specially conclusive, I think, in which the larvæ were introduced directly into a loop of the jejunum, separated from continuity with the rest of the intestine by making a " Vella fistula."

Moreover, it is quite remarkable that fed larvæ of *Uncinaria stenocephala* —but perhaps not of *Ancylostoma caninum*—enter the glands of the mucosa of the stomach and intestine, in order to return apparently after some days to the lumen of the bowels for further development (fig. 5). A similar phenomenon is described for the larvæ of *Trichuris vulpis*, *Heterakis gallinæ*, *Trichostrongylus* and *Oesophagostomum columbianum* and we can interpret it with Monnig " as a remnant of a former migratory process " of the larvæ just as the presence of branchial clefts in the human embryo is morphologically a " remnant " of organs once useful to his ancestors.

I think it is quite probable that the larvæ of the ancestors of *Ancylostoma* infected only through the skin *via* the lung, and the fact that larvæ with primitive buccal capsules are found, apart from the intestines, only in the lungs, indicates perhaps that originally the full development of *Ancylostoma* could be completed in the air passages as is the case even now with *Strongyloides stercoralis*. The first step of the parasitism of this " skin infecting " group of nematodes may be represented by *Rhabdias bufonis*, closely related to *Strongyloides* but living not in the intestine but exclusively in the lung of the frog, and apparently under conditions very similar to those of free-living nematodes.

The larvæ of *Trichinella*, *Hepaticola* and the other Trichotrachelidæ wandering from the intestine with the circulation to their " specific organ " where they are arrested for further development—apparently by a " chemotaxis "—cannot be discussed here in detail. It is true that the larvæ of *Trichuris* mature in the intestine without leaving it as shown by myself (Fülleborn, 1923A) and corroborated by Hasegawa; yet according to the Japanese author the young larvæ (as mentioned before) for some days invade the glands of Lieberkühn, apparently as " a remnant of a former migratory process." Moreover, the mouth-spear that I found in the youngest larvæ of all the investigated Trichotrochelidæ, including *Trichuris* and *Trichinella* (Fülleborn 1923B) reminds one a little of the spear of *Dorylaimus* and related forms, giving perhaps a hint as to the group of free-living nematodes from which these parasites may have descended.

As found by Stewart (1916) the larvæ of *Ascaris lumbricoides* swallowed in matured eggs wander, after hatching in the gastro-intestinal tract, from the intestine, *via* liver, lung and trachea back to the intestine where they reach sexual maturity, just as shown by us for fed *Strongyloides* larvæ. First they penetrate the intestinal walls, and some of them are found in the lymphatic glands. But the great majority, according to the experiments of Ransom & Cram and myself (Fülleborn 1921B and 1925) is transported with the blood of the portal vein to the liver and from there through the vena hepatica to the right heart and the lung. The few larvæ found in the abdominal cavity of infected animals certainly do not play such an important rôle in the infection of the liver, or of the lung through the diaphragm, as suggested by Yoshida (Fülleborn 1921B). Moreover, my experiments suggest that the majority of the larvæ accumulating in the abdominal cavity in the later days of the infection have broken out only accidentally from the infected liver or from blood vessels (Fülleborn 1922A and 1925, p. 34).

The perforation of the lung capillaries by the larvæ causes, as is well-known, small hæmorrhages and broncho-pneumonic processes which, by obstructing the bronchioles, seem to retain many of the larvæ. After the absorption of the exudates in about a week, however, the air passages become passable again and—as I think—the liberated larvæ are then evacuated by the ciliated epithelium up the trachea into the pharynx.

In the meantime the larvæ, originally only 260μ long, have increased

in length in the lung to 1 mm.—2 mm., and are also preparing for the second ecdysis, not casting the old larval skin apparently until they have reached the intestine. For theoretical reasons it is remarkable that *Ascaris* larvæ, not only in the lung but also in other organs and even under the skin, can increase considerably in size (Fülleborn 1925, p. 35 footnote).

As mentioned before, I could show that some *Ascaris* larvæ pass from the pulmonary arteries *via* the pulmonary veins to the left heart. By covering a whole kidney of a living guinea-pig with an india-rubber bag, absolutely impermeable to the larvæ present in the abdominal cavity, it could be proved (Fülleborn 1921B, 1925, p. 65) that the kidneys also are invaded, not merely by the simple penetration of such larvæ—as suggested by Yoshida (1919A and B, and 1920)—but *via* the systemic arteries, where they cause the characteristic bleedings in the uriniferous tubules. Naturally, with the blood stream the larvæ must reach all the organs of the body, and I found them also in the muscles of the body and of the heart, and also in the brain (Fülleborn 1921A) : the reason why the latter is scarcely damaged by the larvæ has already been discussed.

Experiments with *Toxocara canis* (the former *Belascaris marginata*) of the dog, confirmed by other authors (Schillinger & Cram, and Augustine) indicate that with the blood stream the larvæ can apparently reach the placenta also, because they infect the lungs of unborn puppies. In the first days after birth they descend to the intestine, where they reach sexual maturity (Fülleborn 1921C). The intestine of the mother dogs, in consequence of " immunity," is usually not infected.

It may be mentioned that the adult *Ascaris lumbricoides* so often found in the liver, seem not to be " remnants " of the larval infection of the organ but to have invaded the liver anew from the intestine *via* the bile ducts.

Lately I came to the conviction that the " wandering " of the *Ascaris* larvæ can be explained on other grounds than by assuming merely hypothetical ancestors, infecting like *Strongyloides* only *via* the skin ; the behaviour of doubtless nearly related parasites seems to offer a better explanation (Fülleborn 1927).

It is evident that, for example, an aquatic bird hardly has any opportunity of swallowing the eggs of the ascarids living in its bowels. But these eggs, with the bird's fæces, can be taken up by a fish in whose liver we find the developed larvæ ; the bird, swallowing such fish, can

infect its intestine with the parasite. It is clear that the larvæ eaten by the fish must escape evacuation with its fæces, but that by entering the internal organs, they avoid this danger and have a good chance of reaching sexual maturity later in the bird. Hence the efficacy of the wandering of the larvæ in the "intermediate" host.

Analogous to the larvæ of the fish's liver are, I think, the far advanced *Ascaris* larvæ of the lung, and they reach this high development not only in man and pig—in whose intestine they can attain sexual maturity—but also in the lung of rabbits, guinea-pigs and perhaps of every mammal.

According to the experiments of Martin, there can be no doubt that—like the bird swallowing the infected fish—a man can infect his intestine with *Ascaris* by eating the larvæ with the lung of a rabbit or, if he is a cannibal, with the lung of a missionary. But such an infective meal has become dispensable because the *Ascaris* larvæ of the man's own lung, in consequence of merely anatomical reasons, are transported by the ciliated epithelium to his mouth. As it seems to me, the *Ascaris* larvæ must wander in the man's body, because the latter is not only the "definitive" host of the parasite, but also one of its many "intermediate" hosts; a biological analogy to this is shown by *Tænia solium* and *Hymenolepis nana*.

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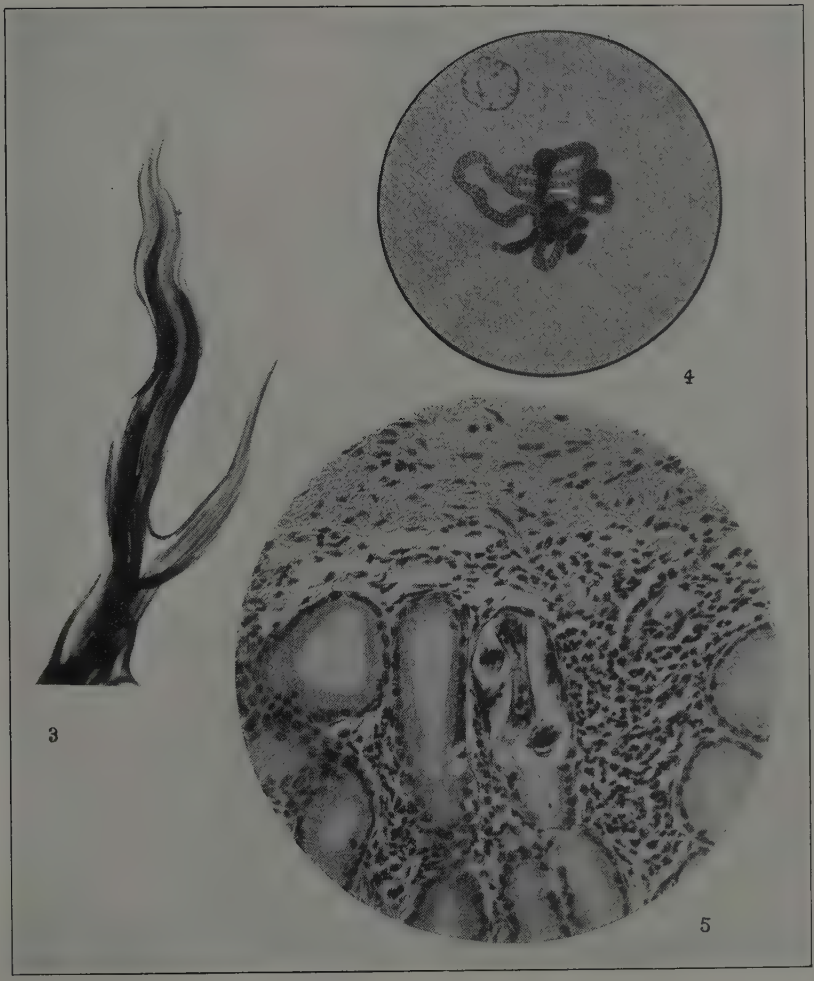
EXPLANATION OF PLATE.

Fig. 3.—The end of a " filament " consisting of many filariform strongyloides larvæ. (Microphot. from a permanent preparation.)

Fig. 4.—Hæmorrhage in a convoluted tubule at the kidney-surface following the arterial-emboli of a strongyloides larva.

Fig. 5.—Section of stomach-mucosa of a dog fed 18½ hours previously with larvæ of Uncinaria stenocephala. (Microphot. 250 : 1 from a hæmatox-eosin preparation.) A curled up larva is seen at the bottom of a glandular tube, where the epithelium is destroyed by it.



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On some New and little-known free-living Nematodes.

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Introduction.

In the course of work undertaken in the preparation of the experimental field plots at Winches Farm it has been of interest to ascertain something about the nematode fauna of the place, especially that occurring in certain heaps of manure made at the farm and afterwards used on some of the plots. With regard to the worms recorded from sheep droppings collected from pastures these were obtained in Baermann extractions, set up by a colleague, in attempts to collect larvæ of parasitic nematodes harboured by the stock. It became necessary for purposes of differentiation to determine the naturally occurring free-living nematodes which were found in great abundance. The worms described in the following pages have been encountered in the course of these investigations. Some are new to science and about others very little has previously been known so that additional information on them is here made available.

Rhabditoides coprophaga gen. et sp. n.

Members of this new genus were extracted from sheep's droppings collected from pastures at Winches Farm in the summer of 1927 and again in September, 1928. In general appearance they resemble species of Rhabditis which occurred along with them in great numbers. The cuticle has rather coarse transverse striations. The buccal cavity is somewhat like that usually found in Rhabditis; its sides, however, are not straight throughout but chalice-shaped anteriorly. The

œsophagus is Rhabditis-like as are also the paired, reflexed female gonads and the single male gonad. The greatest difference from Rhabditis is presented by the male tail, which is without bursal wings, has a tapering spike-like terminal portion and is provided with a number of stout pre- and post-anal caudal papillæ arranged somewhat after the manner of those of the males of Diplogaster species.

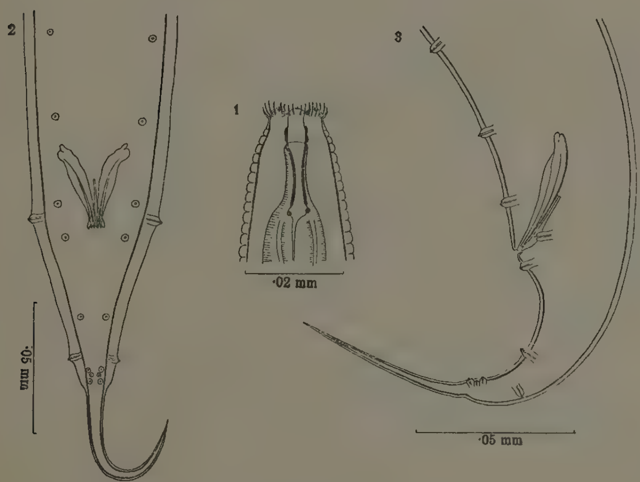
Principal measurements:—Length, females, 1·27 mm. to 1·5 mm., proportions $\alpha = 15$ to 16, $\beta = 7$ to 8·3, $\gamma = 7{\cdot}5$ to 9·6; length, males, 1·12 mm. to 1·15 mm., $\alpha = 16$ to 16·4, $\beta = 7$ to 8, $\gamma = 8$ to 9·6; length of buccal cavity plus pharynx, 0·024 mm.; spicules, 0·03 mm.; gubernaculum, 0·015 mm.; vulva, 43 to 49·6 per cent. of body length from anterior end.

The body tapers gently towards the anterior end. There are six rounded lips each provided with a small papilla. The head is encircled by a ring of delicate bristles which arise laterally on the cuticle at the base of the lips and curve outwards and forwards. There appear to be about 32 of these in all, but they are very difficult to count owing to their delicacy and disposition. The mouth is central and leads into the chalice-shaped buccal cavity which is about one third as wide as the head and about as deep as wide. Its walls are refractive, straight on the inner face but thickened on the outer. It connects behind with the pharnyx, the walls of which stand closer together than those of the buccal cavity and may be gently incurved towards each other or practically parallel (fig. 1). That this part is really pharyngeal is shown by the fact that it is surrounded by a forward continuation of the œsophagus as far as the base of the buccal cavity where the junction of the two regions is marked by a faint transverse line. The walls spread out a little at the posterior end and appear as if broken or very thin and on either side there is a circular refractive dot as in Rhabditis. The œsophagus is in two parts and shaped as in Rhabditis. The first part is muscular and ends in a distinct swelling. It is followed by a narrow neck which expands into the second œsophageal bulb, in which there is a central valve apparatus, the inner faces of which are serrated. The nerve ring crosses the neck quite close to the second bulb and the excretory pore is just posterior to the nerve ring. The musculature of the first part of the œsophagus is supported by longitudinal cuticular fibres.

The intestinal wall is usually densely packed with dark brown fatty

globules. The posterior lip of the anus is rather prominent in the female. In the latter also the body narrows rather sharply just behind the anus and tapers to a finely pointed tail on either side of which there is a small papilla extending from the body-wall to the cuticle.

The vulva is situated a little anterior to a point midway between the head and the tip of the tail. Its lips are rounded and rather prominent.



Rhabditoides coprophaga gen. et sp.n.

Fig. 1.—Head end under high magnification.
Figs. 2 and 3.—Tail region of male in ventral and lateral view respectively.

On either side of the vagina, occupying a lateral position, there is an oval gland having a finely vacuolate appearance. They do not appear to be receptacles for spermatozoa. The gonads are paired, opposed and reflexed, and each uterus usually contains several segmented eggs. Each leads distally into a rounded receptaculum seminis, generally found crowded with sperms, and this connects with the oviduct which occupies the bend of the organ and leads to the ovary. The anterior gonad reaches to about

three-quarters of the distance between the beginning of the intestine and the vulva and the posterior one to about the same distance between vulva and anus.

The male gonad is single and extends almost to the end of the œsophagus, where it is reflexed for a short distance. The male tail region tapers slightly from a point some distance anterior to the anus. Behind this it narrows rapidly and ends in the form of a long spike. It is usually curved ventrally and has no bursal wings as in the majority of Rhabditis species.

The spicules are paired and taper towards the points which are split for a short distance. The body of each spicule is gently curved; the ventral edge is winged and the dorsal border fairly stout. The anterior end of each is rounded and notched. The gubernaculum is about half as long as the spicules. When seen ventrally it is found to be shaped like an inverted Y with the stem away from the anus. There are ten pairs of stout caudal papillæ arranged as shown in figs. 2 and 3. Two pairs are lateral; one of these being just pre-anal and the other post-anal a short distance from the base of the tail. The other eight pairs are sub-ventral in position; three are pre-anal and five post-anal. Of the latter, three pairs form a compact group very close to the mid-ventral line at the base of the final caudal spike, as in many Diplogaster species, and of the other two pairs, one is immediately post-anal and the other a little more than halfway between the anus and the base of the terminal spike.

Systematic position.—The principal differences between the new genus and Rhabditis are (1) the chalice-shaped buccal cavity; (2) the presence of numerous circum-oral bristles; (3) the absence of bursal wings and (4) the arrangement of the male caudal papillæ.

Rhabditis pseudoxycerca n.sp and R. oxycerca de Man, 1895.

Both of these species have been obtained from the same heap of pig-manure; the new species in the extracts, made by the Baermann method, in June and July, 1927 and in September, 1928, de Man's species was obtained from the pig-manure extracted on the last-mentioned date and also from rotting coco-nut palm stem sent from Trinidad to this Institute and examined in September, 1928.

In general appearance, as seen under low magnification, both species are similar to each other in that they are rather stout in comparison to the length, have thick hyaline cuticle and a blunt, rounded tail through which a pointed process protrudes. In the males also of both species a wing-like bursa is absent and the cuticle of the posterior end is merely inflated and supported by a number of papillæ which are differently and characteristically arranged in each. The new species differs from de Man's also in the following features which are dealt with in detail later. The buccal cavity is not straight throughout but has a dorsal bend towards its lower end, the œsophagus is differently shaped and the male tail carries ten pairs of caudal papillæ instead of eight pairs in *R. oxycerca*. The writer can confirm de Man's account of the latter; the worms examined agree in all details with those of the original description. For the sake of brevity and clarity the two species are dealt with side by side in the present paper.

Principal measurements :—*R. pseudoxycerca*, length, female, 0·96 mm. to 1·2 mm., $\alpha = 12{\cdot}7$ to 20, $\beta = 3{\cdot}84$ to 5, $\gamma = 16{\cdot}6$ to 19; length, male, 0·78 mm. to 1·07 mm., $\alpha = 13$ to $15{\cdot}3$, $\beta = 4$ to $5{\cdot}5$, $\gamma = 13$ to 21, spicules, 0·045 mm., gubernaculum, 0·023 mm., vulva, 56 to 59 per cent. body length from anterior end.

R. oxycerca, length, female, 0·8 mm. to 0·88 mm., $\alpha = 13$ to $14{\cdot}8$, $\beta = 3{\cdot}5$ to $4{\cdot}4$, $\gamma = 27$ to 29; length, male, 0·77 mm. to 0·85 mm., $\alpha = 14{\cdot}2$ to $15{\cdot}4$, $\beta = 3{\cdot}4$ to $3{\cdot}7$, $\gamma = 19$ to 21, spicules, 0·032 mm., gubernaculum, 0·013 mm., vulva, 55 to 58 per cent. body length from anterior end.

The cuticle is transversely striated in *R. pseudoxycerca* and the striations are more easily seen than in *R. oxycerca* in which they occur, according to de Man, on the second layer of the thick cuticle, and are difficult to discern when the latter begins to separate from the body-wall soon after death and under the pressure of a coverslip. *R. pseudoxycerca* has six small rounded lips separated from one another by shallow depressions. Each lip carries a small papilla. The head region is shaped like a truncate cone and is limited posteriorly by a slight encircling groove behind which the body gently swells outwards. At the base of each lateral lip can be seen the elliptical opening of the amphid.

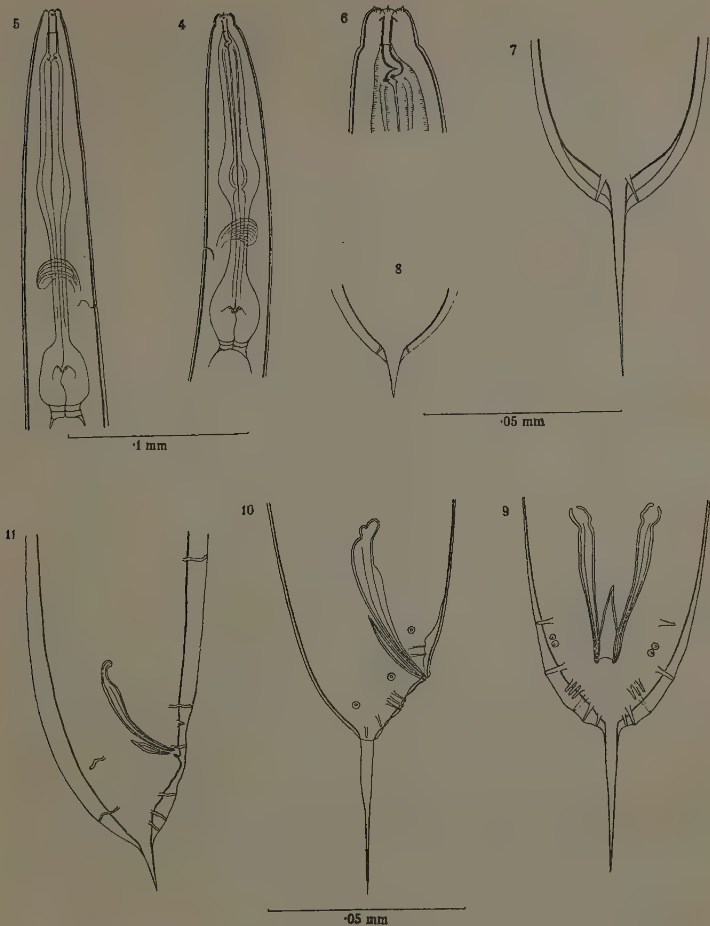
When seen in lateral view, the walls of the buccal cavity run parallel to each other for some distance and then suddenly bend dorsally towards

the lower end with a corresponding inward curve of the ventral wall, followed by a return to the mid-longitudinal line at the connection with the œsophagus. At this point there is an irregular thickening of the cuticular lining as occurs in many species of Rhabditis. As figs. 4 and 5 show, the buccal cavity of both these species is crossed by a faint line at about half its length which marks the forward extent of the ensheathing attachment of the œsophagus. The latter is shaped as shown in fig. 4 and that of *R. oxycerca* in fig. 5. From these it can be seen that the terminal swollen end of the first part is more prominent in the new species than in de Man's. The neck leading to the final bulb is shorter and expands more gradually to the bulb than in *R. oxycerca* where the neck is rather long and the final bulb arises as a sharp angular swelling. A prominent feature also of the œsophagus of *R. pseudoxycerca* are the cuticular fibres running through the neck, the median bulb and the anterior muscular part. They are more clearly seen in this species than in *R. oxycerca.* The nerve ring and excretory pore are situated as shown in figs. 4 and 5.

The female tail is bluntly rounded immediately behind the anus and the cuticle is here seen to consist of two layers. A long tapering spike or process, having its origin in the posterior body-wall, passes into and apparently through the cuticle at the extreme end of the body just as in *R. oxycerca.* It is much longer than in that species as the accompanying drawings show. The male tail carries a final process of the same length and shape as that of the female.

The vulva has rounded lips and is found a little behind the middle of the body. The gonads are paired, opposed and reflexed as in *R. oxycerca.* In the latter species de Man records the presence of coarsely granular glands one on either side of the vagina into which they open by rather long ducts. The writer has also found these glands in the specimens of *R. oxycerca* which he has studied. They appear to be made up of a morula-like mass; the ducts leading from them were not, however, seen. Search was made for similar glands in females of *R. pseudoxycerca* but none were found. In this species there appear to be one or two segmented eggs at a time in each uterus. In *R. oxycerca,* de Man speaks of the females being viviparous, but the writer has not found this condition in his examples.

The male gonad is single and extends almost to the beginning of the intestine where its anterior end is reflexed upon itself for a short distance.



Rhabditis pseudoxycerca n. sp. and *Rhabditis oxycerca* de Man, 1895.

Figs. 4 and 5.—Oesophageal region of *R. pseudoxycerca* and *R. oxycerca* in lateral view.

Fig. 6.—*R. pseudoxycerca* head end under high magnification.

Figs. 7 and 8.—Female tail of *R. pseudoxycerca* and *R. oxycerca* respectively.

Figs. 9 and 10.—*R. pseudoxycerca*, ventral and lateral views of male tail under high magnification.

Fig. 11.—*R. oxycerca*, lateral view of male tail to the same scale as 9 and 10.

The spicules are long and tapering. Seen in lateral view each is knobbed anteriorly with a constriction immediately behind the swelling. The ventral edge is winged as shown in fig. 10. The gubernaculum is rather large and when seen ventrally is triangular in shape with the base underlying the tips of the spicules.

Bursal wings are absent from the tail and the cuticle is merely inflated. It is supported by ten pairs of papillæ, arranged as shown in figs. 9 and 10, compared with the eight pairs and the single medio-ventral found in *R. oxycerca*, fig. 11. Three pairs are pre-anal in position; of these, one is lateral and two are sub-ventral forming a pair close together. Then follow one lateral post-anal and a compact group of three pairs sub-ventral. Behind these come another lateral dorsally directed and two more sub-ventrals both pointing posteriorly. As compared with the caudal papillæ of *R. oxycerca* those of the new species are not so scattered in their distribution and the core of each is straight, not wavy as in that species. There is also no single medio-ventral papilla and none of them are situated as far forward as the first pre-anal in *R. oxycerca.*

Myolaimus heterurus Cobb, 1920.

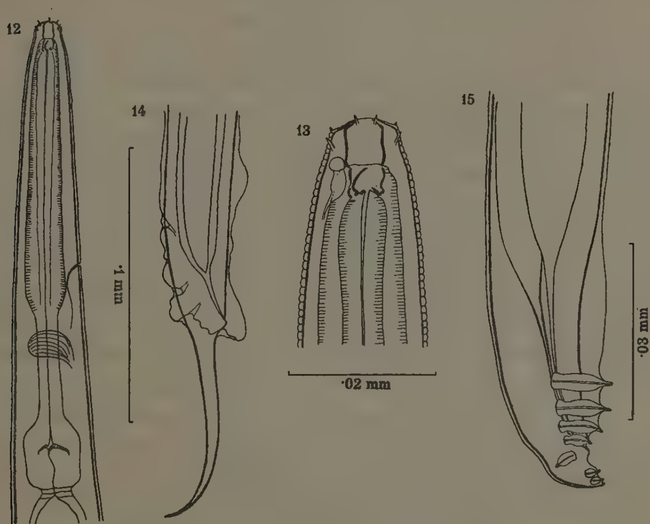
Cobb obtained his examples of this worm, from loamy soil at El Paso, Texas, U.S.A. The writer has found the worms fairly abundantly in water extractions from pig and goat manure which had been lying in a heap at the edge of the orchard at Winches Farm in June and July, 1927 and again in September, 1928. Small numbers were also obtained in December, 1928, in an extraction of a rich dark brown humus taken from under the bole of a fallen walnut tree in the same orchard. It would seem from these findings that the species is normally an inhabitant of the soil of the orchard and favours a rich medium. So far as can be ascertained, the present record seems to be the only additional one since Cobb first described the worms.

Principal measurements :—Length, female, 0·85 mm. to 0·97 mm., proportions, $a = 21$ to 24, $\beta = 4{\cdot}7$ to $4{\cdot}8$, $\gamma = 9{\cdot}7$ to $10{\cdot}6$; length, male, 0·7 mm. to 0·75 mm., $a = 23$ to 25, $\beta = 4{\cdot}4$, $\gamma = 50$, Vulva, 50 to 54 per cent., body length from anterior end, egg, 0·05 mm., long by 0·025 mm. wide. Cobb gives 0·59 mm. and 0·58 mm., as lengths of female and male respectively, so possibly he was dealing with young forms.

In all the specimens examined the cuticle is found to be very loosely

fitting to the body and has the appearance of being sloughed. Cobb remarks that the male specimens examined by him were seen to be moulting. It would seem therefore that this appearance of the cuticle is normal to the worms. Under oil-immersion the cuticle is seen to have fine transverse striations.

Separate lips were not found at the rather flattened head end. The central mouth opening is surrounded by six small labial papillæ and a



Myolaimus heterurus Cobb, 1920.

Figs. 12 and 13.—Œsophageal region and head end respectively seen in lateral view.

Figs. 14 and 15.—Female and male tail respectively. Figs. 12 and 14 are to the same scale.

short distance behind these there are four cephalic papillæ pointing forward and slightly outwards. The writer's observations confirm Cobb's on these. The buccal capsule is longer than wide and consists of two parts, an anterior buccal cavity without teeth and a posterior pharyngeal cavity surrounded by muscle and furnished with a prominent dorsal tooth and a smaller ventral one both arising from the floor of this region, fig. 13.

The amphids are lateral in position (shown rather dorso-laterally in fig. 13). and situated just in advance of the level of the pharynx. The opening of each is almost circular in outline and the chamber somewhat flask-shaped. The mouth cavity, as Cobb says, resembles in many respects that of some Diplogaster species. The appearances presented by the anterior ends figured by him are, however, suggestive of a collapsed condition of the pharynx.

The œsophagus is shaped as shown in fig. 12, where it can be seen to consist of two principal regions. The fusiform anterior part is muscular in structure and carries longitudinal cuticular supporting strands. It is slightly broader posteriorly than anteriorly and narrows behind by sharp angles to the neck region which is of a uniform diameter throughout. The final bulb is well-developed, arises fairly sharply from the neck and has almost parallel sides. It is provided with three central valves each of which is serrated on its inner face. At the junction of the œsophagus with the intestine there are sphincter cells on either side of the lumen.

The nerve ring crosses the neck of the œsophagus in its anterior half and the excretory pore opens well in advance of it just before the first part of the œsophagus narrows to the neck. The female body diminishes suddenly in diameter on the ventral side immediately behind the anus and the tail gradually tapers to a fine point (fig. 14).

The vulva lies about halfway between the anus and the posterior end of the œsophagus. The gonad is single and the ovary starts a short distance in front of the rectum and stretches as a dorsal strand of cells, gradually increasing in width, to a point about three-quarters of the distance from the vulva to the end of the œsophagus. Here it is reflexed and becomes first a rather thin-walled oviduct after which the wall is much thickened and has a muscular appearance. This part is separated by a sphincter from the uterus which seems to contain one egg at a time and numerous spermatozoa. There is also a post-vulvar uterine sac of somewhat variable length but generally about 2 to 3 times as long as the diameter of the body. It also contains sperms. The lips of the vulva are rounded and fairly prominent and the loose cuticle in this region frequently seems to be produced into a vulval flap or projecting hyaline lip resembling that seen in *Hæmonchus contortus* and other parasitic Trichostrongyles.

The male tail is very different in appearance from that of the female

and under low magnification appears rather blunt with little tapering of the body posteriorly. Under high magnification it can be seen that there is a wing-like expansion of the cuticle on each side arising from the body some distance anterior to the cloacal aperture. In the vicinity of the latter each wing is supported by four large column-like papillæ which diminish in size posteriorly and have pointed tips. A fifth pair of papillæ is laterally situated with the points outwardly and backwardly directed. On either side of the tip of the tail there are two pairs of still smaller papillæ. Fig. 15 represents that shape of the tail and the arrangement of the papillæ as found by the writer which agree very well with those figured by Cobb. The body is raised ventrally into a rounded prominence which carries the cloacal aperture and the latter lies at about the level of the third pair of papillæ supporting the wings but it is very difficult to determine how many of these papillæ are pre-anal in position owing to the loose nature of the cuticle. Cobb was doubtful as to the presence of spicules, saying that the appearances he took for spicules might be deceptive and such organs absent altogether. This is actually the case. The writer finds that the duct from the vas deferens is long and very narrrow with a long rectum dorsal to it but no sign of spicules or gubernaculum has been discernible under the highest magnification. The gonad is single and the testis extends forwards almost to the œsophagus where its anterior end is reflexed for a short distance.

Tylopharynx striata de Man, 1876.

This genus which has only one species was described by de Man in 1876 and again in 1884. He obtained his first specimens of both sexes from damp meadow soil and at a later time some growing females from moist earth soaked with brackish water. Since his early accounts further observations on the species have not been published, a point which lends additional interest to the present ones.

Numerous specimens were obtained by the Baermann water extraction method in June and July, 1927, from moist soil taken from a chicken-run in the orchard at Winches Farm and again in September, 1928, from soil rich in organic matter at the base of a manure heap only a few yards from the site of the chicken-run. On each occasion the medium has been very rich in manurial substances ; a point of interest in view of the fact that Bütschli's *Aphelenchus fœtidus*, which the writer has recently

suggested should be transferred to Tylopharynx, was obtained by him from cow dung. de Man (1884) p. 131, remarked on the close similarity of *A. fœtidus* to his Tylopharynx.

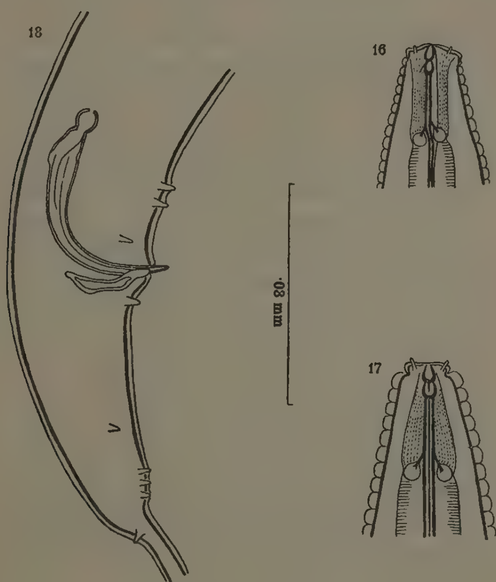
de Man's early observations as to the general shape and size of the worms are confirmed as are also his statements on the arrangement of the male and female gonad, but new light is thrown on the structure of the very complicated buccal apparatus and on the spicules, gubernaculum and caudal papillæ of the male; features of importance in view of the supposed resemblance of the genus to Anguillulina, and certain nearly related genera.

Principal measurements:—length, female, 1 mm. to 1·29 mm., proportions, $\alpha = 33$ to 35, $\beta = 6$ to 7, $\gamma = 3$ to 4; length, male, 0·75 mm. to 1 mm. proportions, $\alpha = 30$ to 32, $\beta = 5$ to 6, $\gamma = 3$ to 4, vulva 40 to 42 per cent. body length from anterior end, spicules, 0·033 mm. gubernaculum, 0·011mm.

The adults of both sex have a rather slender body which tapers a little anteriorly and posteriorly and is provided with a long hair-like tail. The cuticle has fine longitudinal striations, 10 to 12 to the width of the body, which carry the transverse striæ.

The head end is set off by a shallow groove and when seen in lateral view shows a rather conical prominence on each side (fig. 16). In dorsal or ventral aspect these are not seen but an appearance like that shown in fig. 17 is found. de Man (1884), p. 131, says there are no lips, papillæ or bristles on the head. The writer can confirm the absence of lips but has found 4 or 6 small papillæ on the anterior face of the head surrounding the mouth. The latter is very small and leads into the buccal cavity, the structure of which is very complicated. The cuticular lining is strengthened by three rods, one of which is ventral and is thinner and less refractive in appearance than the other two, which are dorso-lateral in position. The ventral is simple and unmodified throughout its length. Each dorso-lateral, on the other hand, gives off towards its base a laterally directed stalk which carries a hollow swelling. Figs. 16 and 17 show the disposition of the various parts and make clear the arrangement of the anterior structures also. The dorso-laterals are joined in front by a bridge-piece shaped like a horseshoe, the forwardly directed tips of which articulate with another similarly shaped portion whose points converge and come close to the mouth opening. A prong-like projection is given

off midway at the base of the most anterior horseshoe and is directed outwards and backwards. The various structures detailed above have been studied in living worms, when moving very slowly, under the oil-immersion. Occasionally the whole buccal apparatus has been seen in movement when it was apparent that the points of the second horseshoe were not rigidly attached to the base of the first one but merely articulating with it. In the same way the dorso-lateral buccal rods articulate



Tylopharynx striata de Man, 1876.

Figs. 16 and 17.—Head end in almost lateral and dorsal view respectively. Fig. 18.—Part of male tail in lateral view. All drawn to the same scale.

with the second horseshoe and when in movement, the base of each horseshoe swings outwards on the forward thrust and back to normal again on the backward movement of the apparatus. There are only two basal swellings, not three, and each is borne on a stalk as a delicate thin-walled vesicle not at all like the basal swellings found in Aphelenchus,

Anguillulina and Heterodera in which the swellings, of whatever size, are enlargements of the posterior end of the stylet wall itself.

The buccal rods are continued posteriorly into the lumen of the œsophagus. The latter is very similar in shape to that found in many species of Anguillulina and consists of three main parts ; a rather narrow first part which expands behind into a prominent muscular bulb following which is the third part, at first a slender neck which gradually swells into a spatulate glandular bulb. The cuticular lining of the muscular bulb is produced into three large crescentic thickenings rather longer and broader than those found in the corresponding bulb in species of Aphelenchus and Anguillulina. The nerve ring crosses the neck of the third part and a little behind it on the ventral surface is the opening of the excretory pore.

In numbers of the worms found in 1927 and again in 1928 the intestine was found to contain large numbers of small rounded or almost cylindroid bodies varying in size from 0·002 mm. in diameter to 0·008 mm. in length by 0·003 mm. in width. In structure they are finely granular with numerous alveoli, but a distinct nucleus could not be made out. They are apparently non-motile as none were seen moving in living worms nor have any special locomotor organs been discerned. Their real nature has not, so far, been determined, *i.e.*, whether they are of animal or vegetable origin, but that they are foreign organisms of some kind or other seems clear from their appearance and distribution in the lumen of the intestine.

The vulva occurs a little in advance of a point midway between the end of the œsophagus and the anus. The gonads are paired, opposed and reflexed and each uterus contains one egg at a time which is laid in a segmented condition.

In the male the gonad is single and outstretched anteriorly with the front end of the testis folded back upon itself for a short distance. The spicules are strongly curved, are knobbed anteriorly with a constriction just behind the head and taper towards the points as shown in fig. 18. There is a keel-like gubernaculum the ventral end of which is closely applied to the sides of the spicules as in species of Diplogaster. There are nine pairs of caudal papillæ having the same distribution as the caudal papillæ in Diplogaster, Odontopharynx and Cylindrogaster. Ia, b and c, consists of three pairs ventro-lateral in position ; Ia and Ib,

are pre-anal and rather close to each other whilst Ic is immediately post-anal ; IIa, b and c, form a small group very close to one another and to the mid-ventral line just at the base of the tail ; IIIa, b and c are laterals ; IIIa is just pre-anal, IIIb is a little more than halfway between the anus and the base of the tail, whilst IIIc is dorso-laterally placed at the base of the tail behind the group IIa, b and c.

Spicules, gubernaculum and caudal papillæ are totally different from the corresponding features in Aphelenchus, Anguillulina and Heterodera. The chief point of resemblance to these three genera is the presence of swellings connected with the posterior end of the buccal apparatus, but as already shown this resemblance is illusory as the swellings in Tylopharynx are only two in number and are stalked vesicles not strictly homologous with the swellings at the base of the stylet in the three genera mentioned. On these grounds, therefore, Tylopharynx should not, in the writer's opinion, be grouped alongside them in any scheme of classification. Micoletzky (1921), p. 432, has pointed out that the buccal apparatus as described by de Man shows some resemblance to that of *Diphtherophora* de Man, 1880, and has included Tylopharynx in his sub-family *Diphtherophorinæ*. This seems a sounder procedure than placing the genus in the sub-family *Anguillulininæ*.

Butlerius butleri gen. et sp.n.

In some rotted banana roots sent to the writer in February, 1928, by Dr. E. J. Butler, Director of the Imperial Bureau of Mycology, there were found a few worms, 5 adult females, 2 adult males and 2 immature females, belonging to this new genus. The material as received was preserved in strong alcohol and the worms were transferred to 70 per cent. alcohol plus 2 per cent. glycerine and finally brought down to and mounted in weak glycerine. The genus has been named *Butlerius* after Dr. Butler and as the worms represent the type species, the specific name *butleri* has been given.

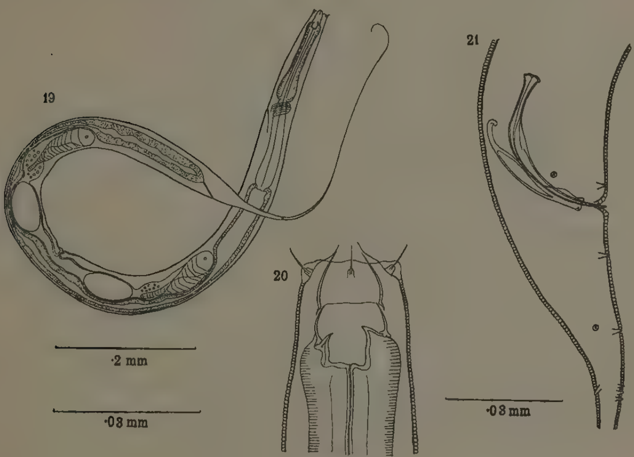
Principal measurements :—Length, female, 1·11 mm. to 1·55 mm., proportions, $\alpha = 18{\cdot}3$ to $23{\cdot}3$, $\beta = 4{\cdot}2$ to $6{\cdot}2$, $\gamma = 3{\cdot}3$ to $5{\cdot}9$, vulva 58 to 64 per cent. of distance from anterior end to anus ; length, male, 0·97 mm. to 1·15 mm., $\alpha = 19{\cdot}1$ to $19{\cdot}6$, $\beta = 5$ to $6{\cdot}6$, $\gamma = 4$ to $4{\cdot}6$, spicules, 0·039 mm. long, gubernaculum, 0·028 mm. long.

The body in both sexes tapers slightly anteriorly and posteriorly, is produced into a long hair-like tail which may be one-third the total length of the body. The cuticle is transversely striated throughout the length of the body but no longitudinal striations were seen. The head is rather broad and anteriorly flattened. In the centre it is raised up in the form of a truncated cone with the mouth aperture in front. There do not appear to be any distinct lips and the walls of the conical region seem rather thin and membranous. There are six cephalic papillæ equally spaced round the head end behind the conical mouth region. Each has a fairly wide basal part which carries a moderately long seta. Amphids were not observed.

As seen in optical section (fig. 20), the buccal capsule is made up of two principal parts; an anterior buccal and a posterior pharyngeal region, a faint line of demarcation between the two being seen running across the capsule. The forward part of the buccal walls form the truncated cone of the mouth. They are relatively thin and hyaline but gradually increase in thickness posteriorly where they articulate with the pharyngeal part. The latter is in the form of a hollow ring with convex walls which are thicker posteriorly than anteriorly. The base of the ring on each side is expanded and is attached to the anterior end of the œsophagus where it curves outwards. The dorsal side is a little in advance of the ventral. The cuticle covering the anterior end of the œsophagus is relatively thick and is produced into two inwardly pointing teeth; one dorsal and the other ventral, the former a little in front of the latter. These two teeth are found on either side of an almost cylindrical hollow let into the anterior face of the œsophagus, a little deeper than wide and having the lumen of the œsophagus leading out of its floor.

The œsophagus has a total length of from 0·2 mm. to 0·275 mm., and has practically the same shape as that of Odontopharynx. It is made up of two parts each of equal length and shaped as shown in fig. 19. The first part is very muscular, rather wide throughout and expands posteriorly almost to a bulbous swelling. The second part is somewhat narrower than the first and gradually increases in width posteriorly, but does not swell to a bulb. It is apparently muscular in structure but under high magnification elements of glandular tissue can be made out lying between the layers of muscle. The nerve ring crosses the second part of the œsophagus immediately after its junction with the first part and the excretory pore lies just posterior to the nerve ring.

Female.—The vulva lies a little more than half the distance from head to anus. Its lips are rounded but not very prominent. The vagina is short and leads into a common uterine chamber with which are connected on either side the paired, reflexed gonads. The forward extent of the anterior one is a little more than halfway to the end of the œsophagus and the posterior one reaches almost exactly halfway between



Butlerius butleri gen. et sp.n.

Fig. 19.—Female worm showing principal anatomical features.

Fig. 20.—Head end in lateral view, high magnification.

Fig. 21.—Part of male tail, lateral view. The lower scale on the left applies to Fig. 20.

vulva and anus. The recurved ovaries do not reach back to the level of the vulva but only about halfway to it. There appears to be one egg at a time in each uterus and in between the latter and the ovary there is a swollen receptaculum seminis.

Male.—The male gonad is single and extends 84 per cent. of the distance from the anus to the beginning of the intestine and its anterior end is reflexed for a short distance. There is a pair of curved spicules and a remarkably large gubernaculum. A single spicule and the gubernaculum, as seen in lateral view, are shown in fig. 21. The anterior

end of the spicule is expanded into a head, posterior to which there is a straight shaft like the handle of a curved knife. It then widens out somewhat to its greatest breadth and from this region gradually tapers to a fairly sharp point. The gubernaculum is more than two-thirds the length of the spicule ; its head end lies close to the dorsal side of the body-wall and is curved inwards towards the spicules in a small expansion or hook. The body of the gubernaculum is hollow and in the distal third of its length it seems to enclose the tapering spicules which pass completely through it. On each side of its ventral end there is a small sharp lateral prominence.

There are a number of caudal papillæ arranged in pairs on practically the same plan as those found on the males of Diplogaster, Odontopharynx, Cylindrogaster and Tylopharynx. Unfortunately both male specimens are not as clear in respect to these structures as could be desired but after careful examination of both under the oil-immersion, the following arrangement has been made out. Ia, b and c, consists of three pairs of ventro-laterals ; Ia is just pre-anal in position, Ib, about the same distance post-anal as Ia is in front, and Ic, a little more than halfway between the anus and the base of the tail. It is possible that there is another pair in this series pre-anal in position situated at about the level of the head of the spicules but owing to the rather flattened state of the worms and the crinkling of the cuticle here their presence could not be made out with certainty. IIa, b and c, consists of the usual three pairs forming a compact group close to the mid-ventral line just at the base of the tail. IIIa, b and c, are the laterals ; IIIa, is just pre-anal, IIIb, is post-anal about halfway between anus and base of tail, whilst IIIc is dorso-lateral at about the same level as group II.

Systematics.—At first sight the worms were thought to be some species of Diplogaster but on closer examination it was realised that they presented certain features of anatomy not found in members of that genus. The chief of these is the shape of the œsophagus which, although made up of two parts, has not the usual appearance of the œsophagus found in Diplogaster species but is closely similar to that of *Odontopharynx longicaudata* as figured by de Man (1912). Another difference shown by *Butlerius* is in the buccal capsule, the great size and structure of which separate it from Diplogaster. The two fixed teeth guarding the entrance to the hollow cavity in the anterior end of the œsophagus are again different

from the movable teeth found in some species of Diplogaster. The cavity just mentioned is another distinctive feature of the new genus. The blunt anterior end with the mouth on the summit of the truncated cone, the sides of which are without radially segmented leaf-crown elements and the absence of lips mark it off from Diplogaster. Its buccal capsule characters and the shape of the spicules also separate it from Odontopharynx. On all these grounds, therefore, the writer feels justified in creating a new genus for the reception of the worms.

Diplogaster vorax n.sp.

Considerable numbers of this species were obtained in extracts from pig-manure, made by the Baermann method, in June and July, 1927, and in Spetember, 1928. The worms are carnivorous in habit and were frequently seen attacking and devouring other nematodes, ciliated protozoa and tardigrades which occurred along with them in the watery extracts.

Principal measurements :—Length, female, 1·38 mm. to 1·59 mm., width, 0·05 mm., proportions, $\alpha = 27{\cdot}6$ to $31{\cdot}8$, $\beta = 5{\cdot}9$ to $7{\cdot}4$, $\gamma = 1{\cdot}8$ to $2{\cdot}5$; male, 1·1 mm. to 1·24 mm., width, 0·04 mm., proportions, $\alpha = 27{\cdot}5$ to 31, $\beta = 6{\cdot}3$ to 7, $\gamma = 2{\cdot}55$ to $2{\cdot}6$., spicules, 0·04 to 0·042 mm., gubernaculum, 0·02 mm. long.

The body in both sexes tapers but slightly towards the anterior end which, as seen under low powers appears rather flattened and broad. The tail is very long and hair-like in both sexes.

The cuticle is raised up into prominent longitudinal ridges which arise just behind the head region and extend to the beginning of the tail. There are about 20 ridges to the body circumference at its widest part and they become fewer as the body tapers towards the tail. They stand out from the body for a considerable distance and give the cuticle an appearance of great thickness.

Each ridge carries numerous fine transverse striations on each side which do not appear to reach down to the bottom of the comparatively deep grooves between the ridges.

The head end is furnished with six flattened conoid lips on each of which there is a fairly stout bristle-like papilla pointing forwards ; the male has four additional stout, seta-like papillæ situated a little further back and directed outwards and forwards. The amphidial openings are slit shaped, are lateral in position and situated at about the level of the beginning of the œsophagus.

The mouth aperture is wide and the membrane surrounding it is supported by some 20 rod-like thickenings radially arranged around it and resembling in appearance the leaf-crown elements found in this region in certain animal parasitic nematodes. These rods or segments extend as far back as the depth of the buccal cavity and form the strengthening elements of its wall. The anterior end of each rod carries a short bristle or seta as shown in fig. 23.

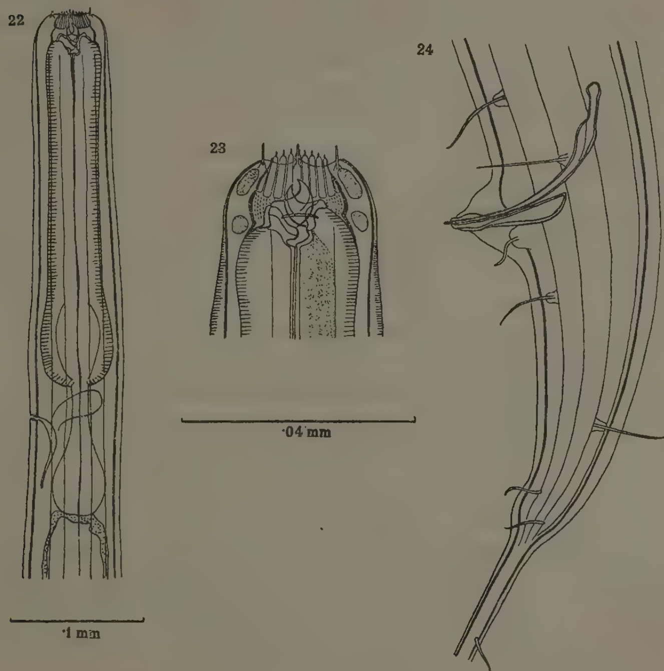
The buccal capsule, as seen in optical section, is wider than deep and is made up of two parts of about equal depth. The first part has been dealt with already so far as the nature of its wall is concerned. In optical section it is convex and the wall on each side is pinched in just before it attaches to the second part. The wall of the latter is a little thicker than the first part and, as shown in fig. 23, it curves outwards and is attached to the outside of the œsophagus. The inner surface of the second part is encircled by six or eight rows of very small backwardly directed denticles.

There are two large teeth, one dorsal and one ventral, attached to the anterior end of the œsophagus. Each is shaped like a cat's claw and is ridged along the convex side. The points are directed forwards and outwards (fig. 23) and the teeth can be actively moved inwards and outwards across the buccal capsule; the fulcrum of each being on the inner side just where the lumen of the œsophagus begins. It is by means of these teeth that the worms attack and puncture their prey. The head is applied to the object, the teeth are pushed forward and by rapid movements cut into the covering membrane and then the contents are sucked out or the object may be torn up still further and swallowed.

The œsophagus is in two parts; the anterior muscular and the posterior glandular. The former is from 2½ to 3 times as long as the latter and is very broad throughout its length (fig. 22). It ends in a distinct swelling the muscles of which are supported by cuticular strands which extend forwards in the substance of the œsophagus and lie about halfway between the lumen and the outer edge. The glandular part consists of a short neck which swells out into a bulb much smaller than the terminal swelling of the first part. A stretch of glandular tissue can be seen extending the whole length of the first part of the œsophagus on the dorsal side and ending at the base of the dorsal tooth. The nerve ring crosses the neck of the second part and the excretory pore lies a little posterior to it.

The intestine calls for no special description. Fatty food bodies are not very abundant in its walls ; a condition probably associated with the carnivorous habits of the worms.

Female.—The vulva is situated practically midway between the end of



Diplogaster vorax sp.n.

Figs. 22 and 23.—Œsophageal region and head end respectively lateral view.
Fig. 24.—Part of male tail, lateral view. Figs. 23 and 24 drawn to the same scale.

the œsophagus and the anus. The gonads are paired, opposed and reflexed ; the anterior and posterior limits of the ovary reaching to about half the distance between the vulva and the beginning of the intestine and vulva and anus respectively. There is one egg at a time in each uterus.

Male.—The single gonad extends anteriorly in the body to about half the distance between the anus and the head end and its forward end

is reflexed for a short distance. The spicules are slender and bowed. The anterior end of each is slightly knobbed, then follows a longish neck with parallel sides which expands to the widest part of the structure and from here there is a gradual tapering to the tip. The gubernaculum at its distal end is shaped like the bow of a boat whilst proximally it is rather thickened where it surrounds the terminal parts of the spicules. The male tail is shown in fig. 24. There are eight pairs of caudal papillæ. They are very conspicuous objects and each is made up of a conical basal portion from the centre of which arises a long seta. Group I, sub-ventrals, consists of Ia, pre-anal, situated practically on a level with the head of the spicules; Ib, immediately post-anal, on a large rounded base, the seta not so long as the others and frequently bent; Ic, a short distance behind Ib. Group II consists of IIa and IIb only close to the base of the tail. Group III, laterals, consists of IIIa which is just pre-anal, IIIb post-anal and situated well down towards the base of the tail, IIIc instead of being on the body proper have got pushed back on to the tail itself and are found a short distance from its base.

Systematics.—In the possession of six circum-oral papillæ, segmental leaf-crown-like elements round the buccal cavity, two large pharyngeal teeth and in the number and arrangement of the male caudal papillæ the worms resemble *D. fictor* Bastian, as described and figured by Cobb (1914). They are, however, of rather stouter build than that species and differ from it also in the much shorter second part of the œsophagus, in the shape of the spicules and gubernaculum and in the fact that the female gonad has a much greater forward and backward reach than in *D. fictor*. The longitudinal ridges of the cuticle also appear to be deeper than in that species.

Feeding Habits.—A word or two may be added on the observations made on the feeding of this species. The cold water extract from the pig-manure was run into Petri dishes and in addition to the worms under description there were numerous other nematodes belonging to Rhabditis, Myolaimus, Aphelenchus, other species of Diplogaster as well as ciliated protozoa and tardigrades. Larval nematodes seemed to be most frequently attacked and killed by *D. vorax*. The latter would swim rapidly through the liquid and then suddenly seize a nematode larva, puncture the cuticle and proceed to suck out the contents of the body. Sometimes nematodes are swallowed whole or large portions are taken into the

intestine as on one occasion the remains of one were shot out from the anus of a specimen and in these remains it was possible to determine the characteristic buccal capsule of another species of Diplogaster. Rounded-up ciliates were frequently seen in the process of being devoured and on several occasions tardigrades were found with *D. vorax* hanging on to them and sucking out their contents. When coming into the vicinity of another feeding worm the approacher seems to become aware of the presence of a possible meal for the swimming slows down and the head is moved from side to side as if seeking the whereabouts of the food. Having located the desired object, a sudden thrust of the head is made with an attempt to get the teeth fixed into it which is not always managed successfully at the first shot. On one occasion a worm was seen to attempt to feed on a nematode larva already held by another worm. A fierce struggle ensued accompanied by rapid lashings of the body and tail of each in their efforts to carry off the object and in the end the original holder kept it. Occasionally also specimens of *D. vorax* were seen to attack each other but in no case did they seem to be able to get a secure hold on the body, the pronounced ridges of the cuticle apparently affording an admirable protection and preventing the teeth of the attacker from penetrating to the body wall.

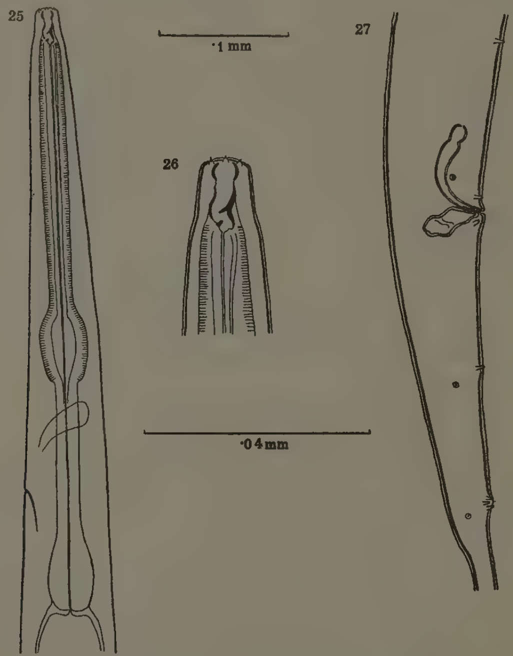
Diplogaster microstoma n.sp.

Several adults of both sex of this species were obtained in the Baermann extracts of pig-manure in June and July, 1927, at Winches Farm. Adults and larvæ were preyed upon by *D. vorax*.

Principal measurements :—Female, length, 1 mm. to 1·34 mm., proportions, $\alpha = 27$ to 28, $\beta = 5$, $\gamma = 3$, vulva, 44 per cent., of body length from anterior end ; male, 0·88 mm. to 0·9 mm., $\alpha = 28$ to 29, $\beta = 4$ to 5, $\gamma = 2\cdot6$, testis extending 60 per cent. of distance from anus to anterior end ; spicules 0·021 mm.

The cuticle has longitudinal striations which carry the transverse striæ. The body tapers considerably towards the anterior end and the head is quite narrow whilst the tail is long and hair-like. There are six small conoid lips round the mouth and each carries a short papilla. The buccal capsule (fig. 26) is longer than wide and is made up of two parts ; an anterior buccal cavity, the walls of which are convex in outline, as seen in optical section, and a posterior pharyngeal part which is longer

than the buccal part and has walls almost parallel to each other. It is separated from the buccal part by a short space where the walls are very thin. On the dorsal wall of the pharynx there is a fairly large tooth the point of which is directed outwards and slightly backwards. At the base of the ventral wall there appear to be two small thickenings or prominences scarcely to be considered as teeth.



Diplogaster microstoma sp.n.

Figs. 25 and 26.—Œsophageal region and head end respectively, lateral view. Fig. 27.—Part of male tail, lateral view. The upper scale applies to Fig. 25, and the lower one to Figs. 26 and 27.

The œsophagus (fig. 25) is comparatively long. It is divisible into the usual two parts; the first region being slightly longer than the second and ending in a distinct swelling somewhat oval in outline and wider in front than behind. The terminal bulb of the second part is rather

small and but little wider than the rest of this region. The nerve ring crosses the neck of the second part towards its anterior end and the excretory pore is found a short distance behind it.

Female.—The gonads are paired, symmetrically opposed and reflexed reaching anteriorly and posteriorly a little more than halfway between the vulva and the end of the œsophagus and the vulva and the anus respectively. There is one segmented egg at a time in each uterus.

Male.—The single gonad is reflexed on itself for a short distance at its anterior end. The spicules are comparatively simple in structure. Each has a rather round head which is off-set behind by a slight constriction following which the spicule tapers gradually to the point. The gubernaculum is shaped as shown in fig. 27. It has an irregular outline and its longer axis is inclined obliquely backwards from the narrower ends of the spicules. No part of it seems to surround the proximal region of the spicules.

There are ten pairs of caudal papillæ arranged as follows :—Group I, sub-ventrals, consists of Ia and Ib pre-anal in position ; Ia being about 0·02 mm., in advance of the heads of the spicules and Ib being immediately pre-anal ; Ic is just post-anal and Id is found about midway between anus and the base of the tail. Group II consists of IIa, b and c, forming a small group very close to the mid-ventral line at the base of the tail. Group III, laterals, consists of IIIa pre-anal and just in advance of Ib, IIIb post-anal and a little posterior to the level of Id, and IIIc dorso-lateral, just behind group II at the base of the tail.

Diplogaster winchesi n.sp.

A few adult males and females of this species were obtained in an extract made from some rubbish, chiefly decaying leaves, taken from an old drain at Winches Farm in July, 1927.

Principal measurements :—Length, female, 1·3 mm. to 1·35 mm., proportions, $\alpha = 26{\cdot}8$ to 27, $\beta = 7{\cdot}4$ to $7{\cdot}5$, $\gamma = 3$, vulva 40 per cent. body length from anterior end ; male, 1·16 mm. to 1·18 mm., $\alpha = 29$ to $29{\cdot}5$, $\beta = 7$ to $7{\cdot}3$, $\gamma = 3{\cdot}5$ to $3{\cdot}8$, spicules, 0·029 mm., gubernaculum 0·012 mm. long by 0·007 mm. deep.

The cuticle has longitudinal striations which carry fine transverse striæ. The body is slender and tapers slightly towards the anterior end and the tail is long and hair-like. The head has six prominent rounded

lips each of which bears a long seta-like papilla. The mouth aperture is central and the sides of the buccal cavity are supported by about sixteen longitudinal ridges having the appearance of striations. At the anterior end of each ridge there is a fine papilla-like prolongation as on the similar structures in *D. vorax.* It can be seen from fig. 29 that each ridge is formed by the inner portion of the cuticular thickenings which form the wall of the buccal cavity. Each of these is shaped rather like a V with the outer limb running into the body wall of the head region and the inner forming one of the ridges just mentioned. The base of the V lies a little more than halfway down the buccal capsule. The wall on each side then thins out for a short distance but becomes thickened again where it is attached to the anterior end of the œsophagus. There are two teeth, one dorsal and one ventral, the broad bases of which are attached to the œsophagus. The point of each is shaped like a small claw but is not nearly so prominent as the teeth in *D. vorax.*

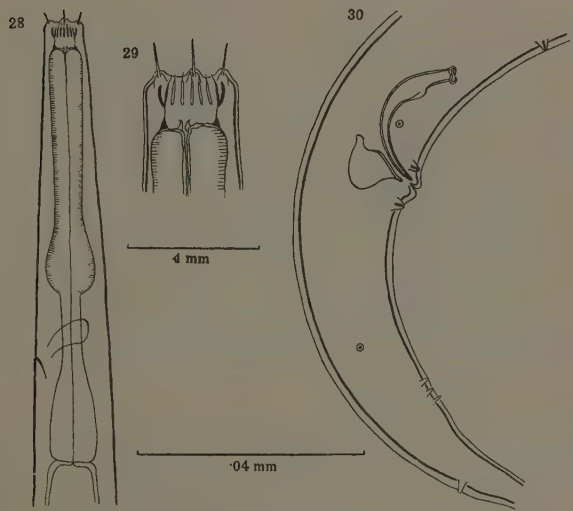
The œsophagus (fig. 28) is in the usual two parts characteristic of Diplogaster; the first part is muscular and is rather longer than the second glandular part. The proportion of the lengths of the two is as 7 to 5. The first part swells out posteriorly into a distinct bulb and the second part after a short neck region becomes spatulate in outline. The nerve ring crosses the neck and the excretory pore is found just behind it.

Female.—The vulva is in advance of the middle of the body. The gonads are paired, opposed and reflexed. The forward and backward extent of the anterior and posterior section is just a little short of halfway between the vulva and the end of the œsophagus and the vulva and anus respectively. There appears to be one egg at a time in each uterus.

Male.—The gonad is single and extends anteriorly to about 70 per cent. of the distance from the anus to the head end. The spicules are strongly curved and each is shaped as shown in fig. 30. having a flattened, ring-like head followed by a fairly broad neck. There is then a prominence on the ventral side giving the spicule its greatest width and posterior to this it tapers gradually to the tip. The gubernaculum is rather short and, when seen in lateral view, shaped like the deep keel of a yacht.

There are nine pairs of caudal papillæ arranged as follows:—Group I sub-ventrals, consists of Ia and Ib pre-anal in position; Ia is situated

about 0·02 mm. anterior to the heads of the spicules, Ib is immediately pre-anal, Ic is just post-anal. Group II consists of IIa, b and c forming the small group close to the mid-ventral line towards the base of the tail. Group III, laterals, consists of IIIa pre-anal a short distance in front of



Diplogaster winchesi sp.n.

Figs. 28 and 29.—Œsophageal region and head end respectively, lateral view. Fig. 30.—Part of male tail, lateral view. The upper scale applies to Fig. 28, and the lower one to Figs. 29 and 30.

Ib, IIIb a little more than halfway from the anus to group II, IIIc dorso-lateral at the base of the tail. In the shape of the gubernaculum, and the number and arrangement of the male caudal papillæ the species resembles *D. asymmetricus*, Steiner, 1927. The dimensions given are, however, larger than for that worm and the buccal cavity is different in certain details from that figured by Steiner.

DIPLOGASTER COPROPHAGES de Man, 1876.

The writer has assigned to this species numbers of worms of both sexes obtained in extracts from sheep droppings collected from a pasture at Winches Farm in June and July, 1927. They present certain points of difference from the worms described by de Man but the resemblances outweigh these and the worms have accordingly been placed in this species rather than in a new one. The male is described for the first time.

Principal measurements :—Length, female, 1 mm. to 1·6 mm., $\alpha = 24 \cdot 2$ to 26, $\beta = 11$ to 13, $\gamma = 3 \cdot 1$ to $3 \cdot 5$, vulva 37 per cent. to 40 per cent. of body length from anterior end; male, 0·75 mm. to 1 mm., $\alpha = 25$ to 27, $\beta = 8$ to 10, $\gamma = 3 \cdot 7$, spicules, 0·026 mm., gubernaculum, 0·01 mm., de Man gave the length of the female as 1·17 mm. to 1·24 mm.

The cuticle has fine transverse striæ and faint longitudinal ones. The body tapers but little anteriorly and the head end is broad and flattened in front when seen under low magnification. The posterior region tapers considerably and the tail is long and hair-like. There are six bluntly conical or rounded lips each bearing a fine papilla. de Man did not figure the lips but shows the presence of papillæ here. The amphid apertures are in the form of transverse slits at the level of the teeth.

The mouth aperture is comparatively wide and leads into the buccal capsule which is shaped rather like a truncate cone when seen in optical section. The bounding wall is made up of two parts as shown in fig. 31. Each part is narrow in front and becomes thicker behind. The first part, buccal, rests on the tips of the second, pharyngeal part and the bases of the latter are attached to the anterior end of the œsophagus. Surrounding the mouth aperture and strengthening the wall of the buccal cavity there are from 16 to 18 ridges having the appearance of leaf-crown elements. This is a point of resemblance to *D. coprophages* as de Man figures this region with divergent striations. There are two moderately large teeth, one dorsal and one ventral, the broad bases of which are attached to the anterior end of the œsophagus. Each tooth is claw-shaped but not so large as the corresponding teeth in *D. vorax*. Judging from their appearance and mode of attachment to the œsophagus they are movable and probably function in the same way as those of *D. vorax* though the writer has not observed this species devouring other animals.

de Man recorded one large dorsal tooth in *D. coprophages* and was doubtful about the presence of ventral teeth. This might be regarded as a point of difference from the worms found by the writer but at the same time too much stress should not be laid on it. The teeth, though large, are not always easily discernible particularly if they happen to be rather retracted, and the worm not lying in a suitable position for observation.

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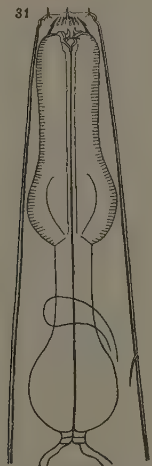

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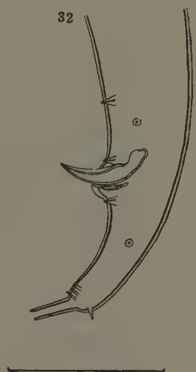


Diplogaster coprophages de Man, 1876.

Fig. 31.—Œsophageal region, lateral view.
Fig. 32.—Part of male tail, lateral view.

The œsophagus is broad and comparatively short. The two parts of it are about equal in length ; the anterior section is very wide and occupies practically the whole width of the body. It swells out into a well marked bulb posteriorly and is muscular throughout. The second section begins as a fairly wide neck and expands into a large bulb. The nerve ring crosses the œsophagus just anterior to the second bulb. As shown in fig. 31. the œsophagus is practically identical in shape with that figured by de Man (1876) Pl. X. fig. 38a, and it is mainly because of this striking similarity that the writer decided to consider his species as being the same as de Man's. It is necessary to point out that de Man gave the

proportion, length of body to length of œsophagus as seven whereas in the worms found by the writer the proportion varies from eight to thirteen. The intestine calls for no special description. Its walls frequently contain dark fatty globules.

Female.—The vulva is in advance of the middle of the body. The gonads are paired, symmetrically opposed and reflexed ; each ovary may extend beyond the level of the vulva. There appear to be three or four eggs at a time in each uterus in large females.

Male.—The male is shorter on the whole than the female and rather narrower. The body tapers posteriorly from some distance in front of the anus and the tail is long and hair-like. The single gonad extends forwards almost as far as the end of the œsophagus. The spicules are rather broad and stout. The head of each is knobbed with a constriction behind as shown in fig. 32. Following this the organ reaches its greatest width and gradually tapers to the tip from which to the point of greatest width there is a wing-like expansion on the ventral side.

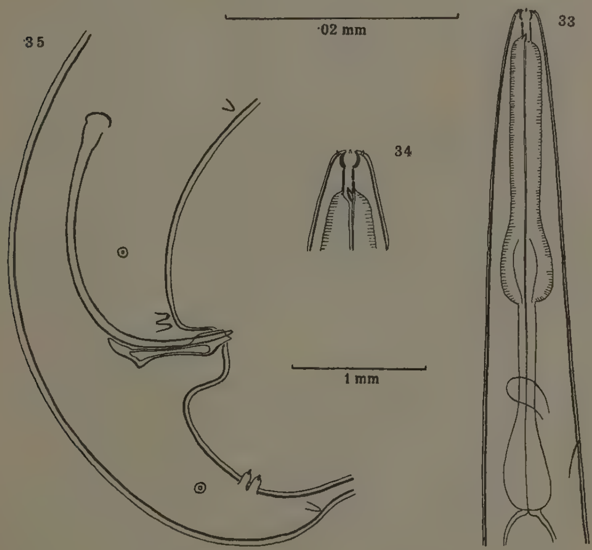
There are nine pairs of caudal papillæ arranged as follows :—Group I, sub-ventrals, consists of Ia, b and c, rather large, conical papillæ. Ia and Ib are pre-anal ; Ia is situated at about the level of the head of the spicules, Ib is immediately pre-anal, Ic is just post-anal. Group II, consists of IIa, b and c forming the compact group close to the ventral line at the base of the tail. Group III, laterals, consists of IIIa pre-anal situated about midway between the level of Ia and Ib ; IIIb is post-anal about halfway between the anus and the base of the tail whilst IIIc is dorso-laterally placed at the end of the body.

Diplogaster gracilis Bütschli, 1876.

The female of this species has a single gonad and the vulva placed posteriorly close to the anus. It was originally obtained from dung and in this connection it is interesting to note that the writer's specimens were obtained in an extract from a heap of goat droppings mixed with hay and straw at Winches Farm.

Principal measurements :—Length, female, 1·35 mm. to 1·39 mm., proportions, $\alpha = 27$ to 28, $\beta = 7{\cdot}3$, $\gamma = 3{\cdot}6$ to $4{\cdot}2$, vulva 73 per cent. to 77 per cent. of body length from anterior end ; male, 1 mm. to 1·07 mm. $\alpha = 35{\cdot}7$, $\beta = 8{\cdot}2$, $\gamma = 7{\cdot}1$, spicules 0·055 mm., gubernaculum, 0·021 mm.

The lengths given above are greater than those given by Bütschli, whose figures for female and male were : 0·9 mm. and 0·8 mm., respectively. The proportions for œsophagus and tail agree fairly well with his which were as follows :—$\beta = 7$, $\gamma = 4$ to 5 for the female and $\beta = 6$, and $\gamma = 6$ to 7 for the male.



Diplogaster gracilis Bütschli, 1876.

Figs. 33 and 34.—Œsophageal region and head end respectively, lateral view. Fig. 35.—Part of male tail, lateral view. The upper scale applies to Figs. 34 and 35 and the lower one to Fig. 33.

The cuticle has faint longitudinal striations which carry fine transverse striæ. The body tapers anteriorly and the head is rather narrow. The mouth is surrounded by six feebly developed lips each of which bears a small papilla. The buccal capsule (fig. 34) is longer than wide and rather tubular in shape. In the anterior third the walls are bi-convex in shape and this region is succeeded by a narrower part in which the walls are

parallel, the dorsal one being made up of two short sections. There is a single small tooth at the base of the dorsal wall but no denticles could be seen on the ventral side. The œsophagus (fig. 33) is composed of the usual two parts and the bulb of the anterior muscular region is wider than the posterior glandular bulb.

Female.—The body maintains about the same width from the beginning of the intestine as far back as the vulva which is prominent in the writer's specimens, and posterior to this it tapers sharply past the anus to the fine hair-like tail. The vulva is only 0·05 mm. in advance of the anus. The vagina is short and leads into a long uterus which connects with the ovary. The latter is reflexed on itself for a considerable distance after reaching to within about 0·2 mm. from the end of the œsophagus.

Male.—The male is more slender than the female and the posterior region of the body is strongly curved ventrally. The anal and spicular orifice is situated on a large rounded prominence. The spicules are long, slender, markedly bowed and have expanded heads. The gubernaculum, which was not shown by Bütschli, is well developed. It is somewhat keel-shaped, as shown in fig. 35 and the proximal end surrounds the terminal region of the spicules.

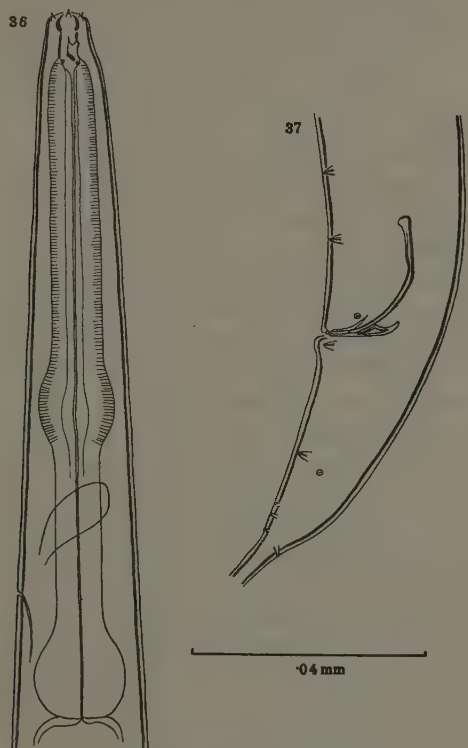
As found by Bütschli there are eight pairs of caudal papillæ arranged as shown in fig. 35. Group I, sub-ventrals, consists of Ia pre-anal placed a little in advance of the head of the spicules, Ib and Ic are very close to each other and are immediately pre-anal. Group II consists of IIa and IIb only, comparatively large papillæ very close to each other and to the mid-ventral line towards the base of the tail. Group III, laterals, consists of IIIa pre-anal at about halfway between the head and the tip of the spicules, IIIb is post-anal and just anterior to IIa whilst IIIc is practically dorso-lateral in position at the base of the tail.

Diplogaster minor Cobb, 1893.

The writer has assigned to this species a few examples of both male and female worms obtained, along with *D. winchesi*, in a water extract made from old leaves, etc., taken from a drain at Winches Farm in July, 1927. Cobb's original specimens consisted of females only from decaying outside sheaths of young banana plants in Fiji.

Principal measurements:—Length, female, 0·8 mm., proportions, $\alpha = 23$, $\beta = 5{\cdot}3$, $\gamma = 3{\cdot}5$, vulva 60 per cent. of body length from

anterior end ; male, 0·73 mm., $\alpha = 26{\cdot}5$, $\beta = 5{\cdot}2$, $\gamma = 3{\cdot}5$, spicules 0·029 mm., gubernaculum, 0·012 mm. The cuticle has very fine transverse striations. The body tapers towards the anterior end and the head



Diplogaster minor Cobb, 1893.

Fig. 36.—Œsophageal region, lateral view. Fig. 37. Part of male tail, lateral view. Both drawn to the same scale.

is comparatively narrow. The amphid openings are in the form of transverse slits and are found at the level of the pharyngeal tooth. There are six small conoid lips round the mouth each of which bears a short pointed papilla. The buccal capsule is a little more than twice as long

as wide and is made up of two parts ; an anterior buccal region, the walls of which, as seen in optical section, are bi-convex in outline separated by a short break in the walls from the second pharyngeal region, the ventral wall of which is straight and the dorsal wall carries a large tooth. The latter projects more than halfway across the lumen and has a forwardly directed point as shown in fig. 36. There is a small cuticular thickening on either side of the entrance to the lumen of the œsophagus. The appearances described are similar to those figured by Cobb (1893) Pl. 4. fig. 3. The œsophagus has the usual two regions. The anterior one is muscular and is rather longer than the second and ends in a swelling. The second part has a neck region of uniform thickness and ends in a small rounded bulb.

Female.—The gonad is single and the vulva is situated a little behind the middle of the body. In Cobb's formula it is indicated as practically at the middle of the body at the same time his fig. 1 shows it as posterior to a point halfway between the anus and the end of the œsophagus and the writer's specimens agree with this.

The single gonad is anterior to the vulva and after reaching a point about wo-thirds the distance between the vulva and the end of the œsophagus it is reflexed on itself and the ovary reaches to within a short distance of the vulva. There is a very short post-vulval, uterine sac. This is a point of difference from *D. monhysteroides* Bütschli, 1874, in which the post-vulval sac is said to extend almost as far as the anus. There is one egg at a time in the uterus.

Male.—The male is similar to the female in the shape of the head, the structure of the buccal capsule and in the shape of the œsophagus. The body tapers gradually behind the anus and ends in a hair-like tail. The gonad is single and extends a little more than halfway from the anus to the end of the œsophagus. It does not appear to be reflexed anteriorly. Each of the slender spicules has a rounded head followed by a rather long straight neck region, but little narrower than the head, and then tapers gradually to the point. The gubernaculum is somewhat wedge-shaped and partly underlies and partly surrounds the terminal region of the spicules as shown in fig. 37.

There are ten pairs of caudal papillæ arranged as follows :—Group I, sub-ventrals, consists of four pairs, Ia and Ib being pre-anal and Ic and Id post-anal in position. Ia is situated a short distance in advance of

the head of the spicules and Ib practically at the level of the beginning of the neck of the spicules. Ic is immediately post-anal and Id a little more than halfway between the anus and the base of the tail. Group II consists of three papillæ IIa, b and c, very close to the mid-ventral line at the base of the tail. Group III, laterals, consists of IIIa just pre-anal in position, IIIb is post-anal and just behind Id, whilst IIIc is dorso-laterally placed at the base of the tail.

Systematics.—In addition to *D. minor* Cobb there was one other species requiring consideration from the systematic point of view, namely, *D. monhysteroides* Bütschli, 1874. In both of these, only the female worm had previously been described and in both the gonad is single and anterior to the vulva. The length given by Bütschli for his species is 0·79 mm. which is practically identical with that given by the writer for the females found by him, 0·8 mm. The proportions $\alpha = 26$ and $\beta = 6$ as calculated from Butschli's figures agree fairly well with those given by the writer. Cobb gives the length of *D. minor* as 0·5 mm. It became a question, therefore, of deciding to which of these two species the writer's forms should be assigned. *D. monhysteroides* was ruled out, in spite of the agreement in length and proportions, because of the presence of a long post-vulval sac whereas in the writer's specimens, as already mentioned, this organ is short and very similar in appearance to that figured by Cobb in his fig. 1. Another point which determined the placing of the worms in *D. minor* was that the head end bears a strong resemblance to that shown by this species as depicted by Cobb in his fig. 3 in which the dorsal tooth is shown.

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The Potato-Root Eelworm in Lincolnshire.

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Introduction.

In 1924, Morgan visited South Lincolnshire to investigate the part played by the potato-root eelworm (*Heterodera schachtii*) in the serious failure of the local potato crop: a note on the investigation appears in this Journal (1925, p. 185). A second visit was made in 1925, as a result of which certain conclusions regarding manuring and the rotation of crops were put forward (this Journal, 1926, p. 49). Towards the end of 1925 the second writer (Peters) made a hurried survey with the object of seeking a possible correlation between the distribution of eelworm cysts and the hydrogen ion concentration of the soil. Such a correlation was actually demonstrated (this Journal, 1926, p. 87) in the case of a selected field for which Morgan had complete data of cyst-counts. The question thus arose as to whether any clear correlations could be shown to exist between the three factors: concentration of cysts, pathological condition of the crop, and reaction of the soil. In other words, was the eelworm associated with the damage? and was it controlled (and therefore controllable) by the effective acidity of the soil? To decide these questions might be a matter of considerable importance, particularly if the eelworm was found to be responsible for the damage, for affirmative answers would open the way to effective treatment, by the adequate liming of soils for instance. With this end

in view the writers carried out extensive tests (in contradistinction to the somewhat intensive tests of the previous years) in the summer of 1926. Well over a hundred samples were collected and brought back to the Institute of Agricultural Parasitology (St. Albans), where cyst-counts and hydrogen ion determinations were performed. Attached to each sample were notes on the pathological condition of the plants in its vicinity, so that data were available for the three variables between which correlation was sought. These data were worked out, and some preliminary experiments had been started on the hatching of the eelworm cysts, when both writers took up new work of a different nature. The eelworm problem was at once handed over to Miss M. J. Triffitt who has since been working at it. Now that her work has reached a stage when some of her data can be published, it seems an opportune time for the writers to present the results of their investigations in 1926, thus linking the old work with the new.

The present paper, then, contains notes on the technique employed and a discussion of the results obtained in investigating possible correlations between the pathological conditions of South Lincolnshire potatoes, the concentration of eelworm cysts in the soil, and the reaction of the soil.

The facilities placed at the writers' disposal by the Principal and Staff of the Kirton Agricultural Institute have been greatly appreciated; thanks are also due to many of the local landowners who readily permitted sampling and freely answered questions regarding the history of their crops. Finally, the writers are very grateful to Professor M. Greenwood, F.R.S., who has kindly read through the manuscript, and given them the benefit of his advice in the matter of statistical methods and terminology.

Technique.

Collecting and Sampling.

As already noted, the earlier investigations were of an intensive nature: complete data were sought from one or two selected plots. The Variety-trial field laid down by the Kirton Agricultural Institute is a case in point. Here, Morgan was unable to reveal any definite correlation

between cysts-count on the one hand and pathological condition, variety of potato, or manurial treatment on the other. He did show, however, that the concentration of cysts followed a fairly even gradient diagonally across the field (1925, p. 187). On this same field Peters was able to show a similar gradient in the reaction of solutions of Morgan's soil samples, such that the maximum cyst concentration occurred in a sample of maximum acidity (*i.e.*, minimum pH-exponent). On this particular plot, then, there is a correlation between cyst-count and reaction, but none between cyst-count and pathology. It would be vain to generalize from a single plot, however, as was shown in the case of a few miscellaneous samples from scattered plots which failed to show the reaction correlation (1926, p. 112). Some of the pH-values and cyst-counts for these miscellaneous samples may be given here (see Table I). There are only two samples for each field, from the neighbourhood of poor and good plants respectively, and only a single cyst-count is available for each, but they serve to show that hint of correlation

TABLE I.

Poor—					
Cyst-count	43	40	44	64	—
pH-value	6·5	6·2	5·6	6·25	6·5
Good—					
Cyst-count	6	14	2	10	—
pH-value	6·2	6·2	5·6	6·4	6·7

Table I. Pairs of samples, each pair from poor and good patches on one field, compared in vertical series.

between cyst-count and pathology which made the present investigation seem desirable. Pairs of samples from the same field are shown in vertical series in the Table. In the four pairs of samples for which cyst-counts are cited it will be seen that poor plants are associated with many cysts, and *vice versa* : of the five pairs of pH-values the expected correlation between high cyst-count and low pH-value obtains only in the last two, there is a reverse correlation in the first, and the other two show no correlation at all. Thus conclusions which might have been drawn from the Variety-trial field tend to be reversed.

The necessary intensive work having been done, the writers decided that the time was ripe for data on an extensive scale. Accordingly the method of approach in the 1926 investigation was quite different : widely separated fields were sampled. In a given field the bad patches

were noted and a series of samples was taken along a line crossing the patch. Each series thus yielded samples associated with plants whose pathological condition varied from good to bad and back to good again. The investigation took place in June when only a few of the Early potatoes had been lifted, and when the condition of the foliage was most easily seen.

The standard method of taking a sample was as follows. A trowel-full of soil was taken from 12 random points within a circle of about 8 feet radius, the soil was mixed and a single trowel-full retained in a numbered bag of tough paper. For the purposes of cyst-counts at least, the surface soil is far from homogeneous, and this method effectively smoothed out the grosser inequalities. From this sampling centre about 12 paces were taken to the next, where a second sample was secured in like manner. The actual number of paces varied, in different series, with the size of the bad patch, in such a way that about five samples were obtained for each patch. At the end of a day's collecting the bagged samples were brought back to the Kirton Institute. After thoroughly mixing the soil in a given bag, about 10 cc. were placed in a cleaned glass-stoppered bottle, two drops of toluene were added and the stopper was sealed on with paraffin wax. These 10 cc. samples were intended for hydrogen ion determinations: the toluene was added to check biological changes which might conceivably affect the reaction, and the bottles were sealed to prevent drying of the soil. The effects of adding toluene and of drying the soil were controlled, as will be seen below in the section on "Hydrogen Ion Determinations." The remaining soil was returned to its bag and retained for cyst-counts.

Cyst-Counting.

The method previously employed for determining the number of cysts in a given sample of soil has already been fully described by Morgan (1925, p. 186). A single count was made from each sample, thus introducing a considerable and undetermined error. Moreover, the counts were related to a *volume* of soil, which procedure would seem less accurate than taking the *weight* of soil as standard. However, time is a consideration in such an investigation, and the field technique (using a soil sampler to a standard depth of 8 inches) applied to some thousands of samples was so lengthy as to necessitate an abbreviated

counting technique in the laboratory. In the present investigation (1926) it was felt desirable to redistribute the times expended in field and laboratory to the advantage of the latter: hence the much more economical use of a trowel instead of a sampler. Some statistical values are now available to show how far these modifications are desirable.

Among the 1925 data are three arrays of counts, all made on the same sample of soil. The arrays represent respectively undried soil measured by volume (16 counts), air-dried soil measured by volume (6 counts) and air-dried soil measured by weight (6 counts). It will be seen that the number of counts in each array is far too small to establish useful statistical values, but these will be recorded nevertheless, partly to introduce the statistical technique employed and partly to give a rough idea of the extent of the variations encountered (see Table II). In the Table, and throughout the paper, σ is the Standard Deviation

TABLE II.

Soil.	Measure.	Counts.	M.	σ	V.
1. Wet ...	Volume ...	16	$45 \pm 1 \cdot 8$	7·0	15·7%
2. Dry	Volume ...	6	$41 \pm 2 \cdot 4$	5·8	14·2%
3. Dry	Weight ...	6	$41 \pm 1 \cdot 4$	3·4	8·4%

Table II. Different counting techniques applied to the same soil sample. For explanation see text.

and represents the square root of the average of the squares of the deviations of an array of counts from the Mean. It has been calculated as follows: $\sigma = \sqrt{\Sigma d^2/n}$, where Σ denotes algebraic summation of the succeeding values, d^2 denotes the squares of the deviations from the Mean, and n is the number of counts. V is the Coefficient of Variation and is given by $V = 100 \cdot \sigma/M$, where M is the Mean; thus V (unlike σ) takes the size of the Mean into account and so gives a measure of the "scatter" of the deviations in relation to it. In the column headed M is given the common arithmetic Mean together with its Standard Error—a measure of the fluctuations likely to be due to mere random variation. In the present case the Standard Error of the Mean has been taken to be: $\sigma/\sqrt{n}$. Generally one atrributes no significance to values less than three times their Standard Errors.

It may be added that only when the frequency distribution approximates closely to the Normal Curve of Error does any such criterion have a precise arithmetical meaning. But, even when the original

distributions are not "Normal," as here, the distribution of *Means* of reasonably large samples tends to normality and justifies the use of the test.

Glancing back at the Table it will be seen that the statistical values, for what they are worth, are definitely in favour of using air-dried soil measured by weight. Before setting out on the present investigation these values constituted the only guide, so they were followed. There are now available three arrays of 50 counts each; the first two belong to the 1925 investigation (from samples taken before and after planting potatoes and mentioned below in the discussion) and are undried samples measured by volume. The third array belongs to the 1926 investigation and represents an air-dried sample measured by weight.

TABLE III.

	Soil.	Measure.	Counts.	M.	σ	V.
1.	Wet ...	Volume ...	50	$40{\cdot}0 \pm 1{\cdot}31$	9·28	23·2%
2.	Wet ...	Volume ...	50	$66{\cdot}7 \pm 1{\cdot}17$	8·27	12·3%
3.	Dry	Weight ...	50	$88{\cdot}5 \pm 1{\cdot}25$	8·81	9·9%

Table III. Statistical values for large counts. For explanation see text.

The three samples came from similar soil, but were taken at different times and have different cyst concentrations, hence they are far from ideal as technique-tests; but because of these differences they are the more useful in showing the close agreement in the standard deviations and standard errors of the means, as can be seen in Table III. This Table shows a standard deviation of less than 10 cysts to obtain, and the various means are subject to a standard error of less than two cysts; the coefficient of variation is naturally higher the lower the mean. The third sample is of some importance since the technique employed in the cyst-counts was that used for all the 1926 samples, except, of course, that 50 counts were not made from each. A rough idea of the accuracy attained in this sample will be given when it is stated that, with a mean count of 88 cysts, three quarters of the counts were within 8 cysts of the mean. Moreover, with very low cyst-counts the standard deviation also tends to be reduced.

The technique for cyst-counting may now be outlined. Three separate counts of each sample were made, and for each count 20 gm. of air-dried soil were used, the soil having been rubbed down but not sieved.

The latter process is wasted on the fine sandy loam of South Lincolnshire (see a mechanical analysis quoted in Peters, 1926, p. 111), but it should be noted that the presence of small stones would introduce a considerable error, whether the soil were measured by weight or by volume, and would render sieving imperative. The cysts were then floated out with water and transferred to a filter paper for counting, as described in Morgan, 1925, p. 186.

The average of three counts for each sample, in place of the single count previously used, gives a more reliable set of values. Some idea of the variation in the three counts can be gathered from Table IV. Here, the statistical values are given for an array of 20 samples taken consecutively from the laboratory notebook. The first values are for an array of the average counts for each sample, the second of the minimum counts, and the third for an array of the maximum counts.

TABLE IV.

Array.	M.	σ	V.
1. Average	$16 \cdot 8 \pm 2 \cdot 7$	12·10	72%
2. Minimum	$14 \cdot 2 \pm 2 \cdot 5$	11·10	78%
3. Maximum	$19 \cdot 7 \pm 3 \cdot 0$	13·54	69%

Table IV. Statistical values for an array of 20 samples to show variation met with. 1, using averages of three counts ; 2, using minimum and 3, using maximum counts.

Hydrogen Ion Determinations.

A full description and discussion of the technique involved in this part of the work may be found in Peters, 1926, pp. 96-105. Briefly (to quote from p. 104), "To 2 gm. of soil were added 10 cc. of twice-distilled water in a 'Pyrex' test-tube. The tube was then stoppered with a waxed cork, and shaken vigorously by hand 20 times every 5 minutes for ¼ hour. The mixture was then centrifuged for 5 minutes at 20 revolutions per second. The resulting centrifugate was diluted with twice-distilled water in the ratio 1 : 9 ; the indicator was added, and aeration resorted to until a constant tint was produced. Colour-matching was then effected in a Comparator of the Walpole type. All tubes used in the Comparator were of practically equal diameter, and were illuminated by daylight reflected from a white surface." One important variation in the present technique was that the soil samples were not dried in any way, and hence sieving was excluded. As promised in the 1926 paper, this factor has been investigated. Nine samples

were divided each into two portions of which one was thoroughly air-dried. The pH-values for the 18 portions are given in Table V. It will be seen that air-drying induces a fairly constant lowering of the pH-value by about 0·1. This change is in the same direction as that observed by Rost and Fieger (1924) to be usual, but is less in extent than that of most of their samples. However, they sampled many different types of soil and, as they state, alkaline soils are subject to greater variation than acid; so that the differences observed in the present case are in general accord with their findings.

TABLE V.

Sample No.	34	35	36	39	40	41	42	59	69
pH. Wet	6·2	6·45	6·1	6·25	6·2	6·3	6·3	6·1	6·15
pH. Dry	6·15	6·35	6·0	6·15	6·15	6·2	6·2	6·0	6·05

Table V. Effect of drying on the pH-value of soils.

Six of the samples were controlled to test the effects of adding toluene. Of these six controls four were without toluene, one had four drops instead of the standard two, and one had ten drops. The resulting pH-values are given in Table VI. From these results it will be seen

TABLE VI.

Sample No.	1	2	3	4	5	6
Drops Toluene	2	2	2	2	2	2
pH-value	6·45	6·15	6·55	6·4	6·25	6·15
Control No.	1a	2a	3a	4a	5a	6a
Drops Toluene	0	0	0	0	4	10
pH-value	6·4	6·15	6·5	6·35	6·25	6·15

Table VI. Effect of adding toluene on the pH-value of soils.

that without toluene there is a tendency for the pH-values to fall by about 0·05; while a concentration of toluene from two drops to ten makes no appreciable difference. The suggestion that the addition of toluene caused a *rise* in the pH-value would be countered by stating first that toluene is not an electrolyte and secondly that if two drops cause a rise of 0·05 then ten drops should cause a still further rise as compared with two, which is not the case.

Statistical treatment of the technique for hydrogen ion determination is neither necessary nor possible, since repeated tests on the same sample invariably gave identical values. The reason, of course, is that any

given sample is homogeneous, as regards its hydrogen ion concentration, within the limits of accuracy of the technique used ; whereas it is decidedly heterogeneous as regards its cyst content.

Pathology.

Of the three factors under consideration the pathological condition of the plants is by far the least satisfactory, since it was not possible to assign to it any standard of measurement. Under certain conditions it might be possible to take the weight of the crop as an inverse measure, but in the present case nothing further than the naked-eye appearance of the growing haulms and of an occasional root was practicable. From this, it was possible to classify the plants in a sampling area as Very Good, Good, Fairly Good, Fair, Fairly Poor, etc. For the purpose of considering the results in bulk (see below) the classes were reduced to three, Good, Fair and Poor, a procedure which resulted in the Fair class being the smallest to a slight extent (24 samples, as compared with 29 Poor and 33 Good) : taking into account the method of sampling it is felt that these three classes, at least, stand for plainly distinguishable differences in the appearance of the plants.

Results.

No useful purpose would be served by tabulating all the results obtained from cyst-counts and hydrogen ion determinations : they must rather be merely summarized in the present section.

TREATMENT IN BULK.

From the very nature of the present investigation (series of samples from different isolated fields) it was not expected that much of value would emerge from a consideration of the results in bulk, nevertheless the effect of such treatment has proved interesting. In this connection the cyst-counts and their relation to pathology will be considered first.

Pathology and Cyst-Counts.

For the purpose of bulk treatment the cyst concentrations of the first 20 samples have been omitted, and this for two reasons : the samples were taken from various fields of the Kirton Agricultural Institute on which the potatoes were uniformly fairly good (thus yielding no variations in pathology), and the average cyst-count for these samples was 63 as compared with 17 for the rest. These experimental fields

of the Institute are under constant scientific supervision and so constitute a class apart from the others: they will be referred to again later. Of the remaining samples 86 are available for treatment, the cyst-count value representing in each case an average of three counts. The frequency distribution of this material gives a somewhat irregular curve, far removed from the Normal. Taking the counts in groups of five cysts, the Mode (most frequent value) actually lies in the group 0–4, while the Mean is in the 15–19 group. The actual statistical values for this distribution will be found in Table IX (p. 75) and may be compared with the values for the "Mean Array" in Table IV (p. 69) which are based on 20 average counts taken at random, instead of on frequency groups for the entire material as in Table IX. The considerable scatter of this array, as compared with the arrays of 50 counts from single samples, is only natural in view of the diversity of the material: what is significant is the low value of the mean and the extremely low value of the mode—the mean being increased by relatively few samples with a high cyst-count.

The material just considered was next subdivided into three classes, Poor, Fair and Good, corresponding to the observed pathological appearances of the plants. Of the frequency curves thus resulting, that for the Poor array is of the Normal type but with a more gradual slope on the right. The mode lies in the group 15-19 cysts, and the mean is 25·6: there are no values in the 0–4 group. The curve for the Fair array is of a similar type but clearly displaced to the left, so that the left limb of the curve is cut short by the ordinate axis. The mode lies in the 10–14 group and the mean is 15·9. The curve for the Good array is situated so far to the left that the left limb is missing, the mode occurring in the 0–4 group and the mean being 9·0. The statistical values for these three distributions are given in Table VII.

A glance at the results seems to show that, even considering the cyst-counts in bulk, there is a tendency for poor plants to be associated with a large number of cysts in the surrounding soil, and *vice versa*. To substantiate this it will be necessary to consider the differences between the means in relation to their standard errors. These differences with their own standard errors are shown in Table VIII. The standard error of the difference has been taken as $\sqrt{a^2 + b^2}$, where a and b are

the standard errors of the individual means. The column headed "Ratio" contains the ratio of the difference to its own probable error; this value should be at least greater than 3 for the difference to be considered significant.

It will be seen that, even between Poor and Fair and between Fair and Good, there are hints of differences in the mean cyst-counts, though they are of somewhat doubtful significance. In this matter, however, some degree of error is necessarily inherent in classifying a particular plant as Fair. As a point of field observation there is far more certainty in differentiating between Poor and Good, and it will be seen that in this case the difference is of indubitable significance.

TABLES VII. AND VIII.

VII. Array.		n.	M.	σ	V.
Poor	... ...	29	$25 \cdot 6 \pm 2 \cdot 1$	11·28	44%
Fair	... ...	24	$15 \cdot 9 \pm 2 \cdot 3$	11·45	72%
Good	... ...	33	$9 \cdot 0 \pm 1 \cdot 6$	9·13	101%

VIII. Arrays.		Differences.	Ratio.
Poor-Fair	... ...	$9 \cdot 7 \pm 3 \cdot 1$	3·1
Fair-Good	... ...	$6 \cdot 9 \pm 2 \cdot 8$	2·4
Poor-Good	... ...	$16 \cdot 6 \pm 2 \cdot 6$	6·3

Table VII. Statistical values for frequency distributions of cyst-counts, subdivided as Poor, Fair and Good.
Table VIII. Differences between means in Table VII.

The writers are driven to the conclusion, then, that after collecting samples from fields taken at random in the Boston district a correlation can be shown to exist between the pathological condition of the potato plants (as revealed in the foliage) and the concentration of eelworm cysts in the surrounding soil. It will be noted that this is directly at variance with the conclusions to be drawn from an intensive study of the Variety-trial field of the Kirton Institute—a point to be raised again later.

Pathology and pH-values.

The method just outlined for dealing with cyst-counts was next applied to the pH-values obtained from the various samples. The frequency distribution curve for the entire set of values (i.e., omitting considerations of pathology) is remarkably smooth and follows the Normal type, except for a displacement of the mean to the right, due to a few samples with a relatively high pH-value. During the pH-determinations, values were read to the nearest 0·05 of the pH-scale,

and these values have been taken in groups of two for distribution purposes. Actually an attempt was made to treat each unit value (0·05) as a group, but this revealed a regrettable predilection, on the part of the writer concerned (Peters), for round numbers—hence the necessity for grouping pairs of units. The mode of the distribution lies in the group 6·15–6·2 at about 6·17, and the mean at 6·24: the other statistical values are given in Table IX on page 75. The displacement of the mean to the right of the mode is possibly due to the presence of lime on a few fields, although the practice of liming is not common. Detailed examination of the samples tends to bear this out since on two fields the high pH-values were uniformly so throughout.

The subdivision of this material under the pathological heads Poor, Fair and Good yields three distributions of the same type as that for the total. All three, however, have practically identical means: such differences as are found are actually less than their own standard errors and are thus chance differences due to the limited extent of the material.

Hence it can be stated that there is definitely no marked correlation between the pathology of the plants and the hydrogen ion concentration of the surrounding soil, when the material is considered in bulk. So far as it goes, this conclusion is again at variance with that deduced from a study of the Institute field, where no significant variation in pathology was noticeable.

Cyst-Counts and pH-Values.

Considering the vaguely defined nature of the measure for pathology, it might still be possible theoretically to find some correlation between cyst content and pH-value, even though the two correlations with pathology seem to preclude this. The methods used above are inapplicable here. Instead, it is necessary to calculate the coefficient of correlation (here represented by r), a value which varies from ± 1 (according as the correlation is direct or inverse) for perfect correlation, to 0 for no correlation. The value of r and of its probable error, using the actual variates of the entire series of observations, have been computed. The writers find that:

$$r = -\ \cdot 0625 \pm \cdot 073,$$

the coefficient being actually exceeded by its probable error. Hence no definite correlation can be shown to exist.

Under this heading of cyst-counts and pH-values it will be convenient to include some further remarks on the work of 1925 (Peters, 1926) for purposes of comparison. In this case a correlation was shown to exist between the two factors by purely topographical methods, since both cyst-counts and pH-values followed a fairly even gradient, spatially, across the field. Statistical values for the frequency distributions of the two arrays will be found in Table X, and may be compared with those for 1926 given in the accompanying Table IX. Treated in this way, as frequency distributions, there are similarities in the two sets of arrays.

TABLES IX. AND X.

		n.	M.	σ.	V.
IX. 1926.					
	Cyst-counts ...	86	$16\cdot6 \pm 1\cdot38$	12·73	77%
	pH-values ...	97	$6\cdot24 \pm 0\cdot019$	0·192	3·1%
X. 1925.					
	Cyst-counts ...	53	$54\cdot2 \pm 6\cdot6$	47·85	89%
	pH-values ...	53	$6\cdot42 \pm 0\cdot032$	0·232	3·6%

Table IX. Statistical values for frequency distributions (total) 1926.
Table X. The same for 1925 (total). For further details see text.

Since the presence of correlation is claimed for the 1925 values but not proven in the present case, it may be desirable to produce statistical evidence for the former to supplement that derived from purely topographical considerations. Again, using all the variates, the coefficient of correlation has been calculated and found to be:

$$r = -\cdot5633 \pm \cdot064,$$

a value which is nearly nine times its probable error. This may be regarded as a statistical confirmation of the conclusion put forward in the 1926 paper, namely that the particular field in question reveals "An indubitable correlation between pH and cyst concentration" (p. 113).

As regards the comparison of this 1925 value with that for 1926, it will be seen that the difference between the two coefficients of correlation is $0\cdot5008 \pm 0\cdot097$, which, if the two samples are comparable is not very likely to have arisen by mere luck of sampling. The data for the two years are not, perhaps, biologically speaking, strictly *in pari materia*, so that the comparison cannot be pressed too far: all that can be said

with any confidence is that the relation between cyst-counts and pH-values is closer in the case of the 1925 values.

TREATMENT IN DETAIL.

It will now be convenient to consider some of the series of samples in detail so as to illustrate the way in which the correlation between cyst-counts and pathology manifests itself in individual fields, and the general lack of correlation between pH-values and the other variables (see Table XI).

TABLE XI.

Series 1.				Series 2.			
No.	Pathology.	Cysts.	pH.	No.	Pathology.	Cysts.	pH.
51.	Good	3	6·2	37.	Good ...	1	6·2
52.	Good	5	6·2	38.	Fair ...	4	6·25
53.	Fair	9	6·0	39.	Poor ...	15	6·25
54.	Poor	13	6·0	40.	Poor ...	21	6·2
55.	Very Poor ...	30	6·1	41.	Fair ...	25	6·3
56.	Very Poor ...	44	6·15	42.	Good ...	3	6·3
57.	Poor	19	6·2				
58.	Fairly Poor ...	11	6·2		Series 4.		
59.	Fair	8	6·1	74.	Poor ...	37	6·15
60.	Good	6	6·1	75.	Fairly Good	40	6·05
				76.	Poor ...	39	5·95
	Series 3.			77.	Poor ...	21	6·4
71.	Poor	16	6·1	78.	Poor-Good...	13	6·7
72.	Good	28	5·95	79.	Good ...	3	7·9
73.	Poor	28	6·0				

Table XI. Detailed values for representative series of samples. For discussion see text.

Series 1 in the table is at once the longest and the most perfectly correlated series obtained. The second series is typical of the correlation revealed in almost two-thirds of the entire material: correlation is marked but imperfect in the case of a few samples. The third series illustrates absence of correlation and, though small, may be taken as typical of this condition in about one-third of the material. One point requires emphasis: while each of two series may show excellent correlation within itself, yet one may contain a sample (to quote actual values) with 12 cysts associated with Very Good plants and the other a sample with 10 cysts associated with Fairly Poor plants. In other words the correlation is more relative than absolute, and so is considerably masked by treating the results in bulk. Series 4 will be referred to in the succeeding discussion.

Discussion.

The results of the present investigation are chiefly interesting in that they tend to reverse the conclusions tentatively reached from the previous work ; yet at the same time there is reason to suppose that the former conclusions were justified—within the limits of the survey. It is obvious that the problem is a very complicated one : there are evidently many factors in operation of which no account has yet been taken.

The entire absence of correlation between cyst-count and pathology, noted by Morgan (1925) for the Kirton Institute fields, and due to the fact that the plants were uniformly fairly good in spite of the great variation in cyst-counts, is plainly associated with such factors as crop rotation and scientific manuring—factors strongly emphasized by Morgan (1926) but neglected far too much by the local growers. In the present case, omitting the Institute fields, there are only 12 samples (out of 86) with more than 30 cysts per 20 gm. of soil, only six with more than 40 and only 1 with more than 50 : of the 12 samples 10 were definitely Poor and 2 Fair. Yet on the Institute fields (again, in the present investigation) there is a cyst-count of 109 associated with Fairly Good, and one of 76 with Good plants respectively ; and the general average of 63 cysts (as compared with 17 for the others) is associated with quite good plants yielding a fairly satisfactory crop.

It would certainly appear that the presence of eelworm becomes unimportant when due attention is paid to the proper husbanding of the plants. The reason for high cyst concentrations on the Institute fields is not obvious : possibly the manurial treatment benefits not only the crop but the worms also. At all events, the Institute crops can support with little harm cyst concentrations twice as great as those which seem fatal to crops outside : "seem" fatal, because it is by no means clear that the eelworm is responsible for damage even where it is associated with it. Morgan has already noted the prevalence of *Rhizoctonia solani*, and this fungus was commonly observed in the present investigation also. In this matter there is need for research of a different nature. Controlled pot-experiments excluding the fungus and micro-pathological investigations suggest themselves. From bulk

and detailed treatment it is clear that, apart from the Institute fields, the eelworm is at least associated with damage to the plants, and this conclusion may well be held to justify further work along the lines indicated.

The correlation between cyst content and hydrogen ion concentration, found on the Institute field in 1925, may be said not to be obvious in the 1926 samples from other fields, considered as whole as a well as in detail. Nevertheless such a correlation is suggested in one or two cases—a fact completely veiled by the statistical treatment. Series 4 in Table XI (p. 76) is a case in point. Attached to samples Nos. 77-79 in the field notebook is the observation : " Abrupt change along row from bad to good," and it turns out that the change in pH-value is equally abrupt (the final value of pH 7·9 is unique among all samples from South Lincolnshire ; the writers must have struck the site of a former lime dump). From another field where no pathological symptoms were evident, the crop being excellent, two samples were taken ; neither of these revealed a single cyst in the six counts (though a very few were seen on the roots of plants) and the pH-values were abnormally high, 6·6 and 6·8 respectively. In a field on the other side of the road, just opposite this good field, were found very poor plants, cyst-counts up to 21, and pH-values varying around 6·1.

Thus there is a distinct possibility that the eelworm is controlled by a maximum pH-exponent of the soil, somewhere in the neighbourhood of pH 6·8. Unfortunately the range of acidity of South Lincolnshire soils is insufficient to test this adequately : 72 per cent. of the samples taken gave pH-values between 6·1 and 6·3 and only two of them values as high as 6·8 (one having 0 and the other 9 as cyst concentrations). Moreover, it may be that, when an even gradient of acidity across a field obtains, the eelworm does select the more acid part. The Variety trial field of the Institute aside, no such gradient has been found to any marked extent. The only case approximating to it is that mentioned above for Series 4 in Table XI, and here correlation does become obvious.

The evidence already given for a maximum pH-limit of tolerance on the part of the eelworm is strengthened by an observation in Morgan's 1926 paper (p. 51) in which, dealing with the possibility of liming and the prevalence in the district of soils with a " lime requirement," he

says: "The addition of lime in some experiments carried out by the writer shewed no improvement in the crop. It was noted, however, that there was an entire absence of eelworm cysts on a small portion of a field which had a fair percentage of lime present while the remainder was heavily infested and shewed a considerable lime requirement."

Similarly, a minimum pH-value for eelworm tolerance is not manifested in the samples. Only two gave as low a value as pH 5·9 and, while these contained relatively few cysts (10 and 3), they are insufficient as a basis for generalization.

As regards an optimum acidity, a hint is forthcoming from the correlation of cyst-counts, in groups of 5, with pH-values, in ranges of pH-0·1, the statistical values for the two distributions being given in Table IX on page 75. By plotting the mean cyst-counts per unit pH-range against the actual pH-ranges it is found that the maximum mean cyst-count is 30·3 in the range pH 6·35-6·4, the next highest being 22·8 in the range pH 6·45-6·5. The mean counts then decrease somewhat unevenly to 4·5 on each side. This suggests that the optimum pH-value for the eelworm lies rather higher than the mode (about pH 6·15) for the district or, in other words, that the eelworm seems to prefer a soil slightly more alkaline than that most usually met with in South Lincolnshire. Applying identical treatment to the 1925 values the optimum is found to lie in the range pH 6·15-6·2, the mode in this case being abnormally high, in the range pH 6·55-6·6. In view of the irregularity of the material, little weight can be placed on the assumption that there is an optimum as high as pH 6·4; there is nevertheless a definite suggestion of an optimum higher than the lowest pH-values met with, and if this is the case the question of correlation is immediately complicated. There may well be a real correlation between cyst-counts and pH-values, but masked by the statistical treatment here used. For, assuming the presence of an optimum pH-value somewhere within the range dealt with, a direct correlation would exist for pH-values below and up to the optimum, and an inverse correlation above it. However, the available material is too scanty for a decision: the question must be left open for possible future work. Taking everything into consideration, the writers would be going too far in saying that there was absolutely no correlation between the cyst content and hydrogen ion concentration

of the samples. It is probably truer to say provisionally that such a correlation does exist, but to a degree which is very slight except where conditions are especially favourable to its manifestation.

The value of crop rotation as a factor opposing disease has already been stressed by Morgan in his 1926 paper. There he cites the case of a field which gave so bad a crop of potatoes in 1920 that the following rotation was resorted to: Oats, Wheat, Seeds, Seeds (ploughed in), and potatoes again, with peas, in 1925. Even after four years' rotation with crops that are apparently immune to attack from this strain of eelworm, there were 74 cysts per 40 cc. of soil (average of 5 samples) before the potatoes were planted. That these cysts were viable was shown by the cyst-count of 140 (average of 5) after the potatoes were lifted: an increase which is corroborated by two sets of 10 samples each, taken from the potato plot and the pea plot along two rows a couple of yards apart, and giving averages of 64 cysts for the immune peas and 130 cysts for the potatoes. Yet, after an early set-back, the potatoes made good with a yield of 8 tons per acre ("ware")—which seems to show the value of rotation even where eelworm is plentiful, and remains plentiful after the rotation. The case just mentioned may be compared with another in which no rotation was practised. Samples were again taken before planting and after lifting, 50 counts being made on each. These samples are Nos. 1 and 2 in Table III (p. 68), from which it will be seen that the means are $40 \cdot 0 \pm 1 \cdot 31$ and $66 \cdot 7 \pm 1 \cdot 17$ cysts per 40 cc. respectively, giving a significant difference of $26 \cdot 7 \pm 1 \cdot 76$, or an increase of 67 per cent. While neither the percentage increase nor the final total concentration of cysts was so large in this case, yet the crop was a complete failure. Other things being equal for the two fields, this would certainly seem a strong case in favour of the practice of rotation.

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Preliminary researches on Mustard as a factor inhibiting cyst-formation in *Heterodera schachtii.*

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Introduction.

Owing to the failure of the potato crops in South Lincolnshire in areas where the eelworm *Heterodera schachtii* was known to occur, investigations were undertaken by this Institute to determine to what extent the nematode was responsible for the losses incurred, and to discover some method for its control. This work was begun by Dr. D. O. Morgan in the autumn of 1924. In order to gain a general knowledge of the natural conditions under which the nematode occurred, and to obtain some indication of the most promising lines on which fundamental research might be carried out, extensive field observations were made. By this means much data was accumulated which has been of great service in the carrying out of later laboratory and field studies.

In addition to the field observations, accounts of which have already been published, a series of laboratory experiments were begun by Dr. Morgan which were necessarily incomplete when, in 1926, the work was taken over by the present writer. These experiments have been repeated and extended by the present writer and form the basis of much of the subsequent work which has been carried out. It is therefore necessary that some account of this early work be given before later investigations can be described.

Dr. Morgan's Experiments With Mustard.

Amongst the lists compiled by Marcinowski (1909) and Kühn (1881) of plant species particularly susceptible to attack by strains of *H. schachtii* specialised on the sugar-beet, are included both black and white mustard. Owing to its rapid growth, this plant seemed peculiarly adapted for use as a host in experimental work on the nematode specialised on potatoes, and, accordingly, attempts were made to produce an infection by growing white mustard in pots of heavily infected Lincolnshire soil. These experiments were, however, entirely unsuccessful, as in no case were the roots of the mustard attacked by the eelworm. It was thought that, since the strain of *Heterodera* used had become specialised upon the potato, the presence of a growing potato plant in the soil might be necessary to stimulate the larvæ to hatch from the cysts, but that, once they became free in the soil they would attack the roots of any plant known to be susceptible. Accordingly, a further attempt to produce an infection on mustard was made by planting in the same pot both mustard seeds and a potato set. Some months later this pot was examined and the roots of the mustard were again found to be free from cysts, while those of the potato showed a moderate infection. It was noted, however, that the number of cysts on the roots of this plant was very much smaller than the number present on another plant which had been grown under the same conditions in similar soil but without mustard.

In order to determine whether the presence of the mustard had any bearing on the reduction in the number of cysts, two further experiments were set up. Two pots containing infected soil were planted with mustard, and the seedlings, after growing to a height of about three inches were broken up and mixed with the soil in which they were grown. A potato set was then planted in each of these pots, and a control pot of infected soil only was similarly planted on the same day. During growth the plants were kept under observation and showed no symptoms of disease. After seventy-four days the pots were turned out and examined. All showed good root development, but again the number of cysts on the roots of the control plant was considerably in excess of the numbers present on the experimental plants with mustard.

An exactly similar experiment was again set up, and again the plants

appeared perfectly healthy and no difference was noted between them with regard to shoot development.

In this instance it was thought advisable to make an estimation of the number of cysts present on the roots, for comparative purposes. This estimation was made by the following method which has also been adopted in all subsequent cases where counts of the cysts present on the roots have been carried out. The soil in the pot was moistened to ensure the mass adhering together when the pot was removed. The shoots were then cut down to soil level and the contents of the pot were carefully turned out and placed in an inverted position on a tall tripod which could be raised to eye-level. To facilitate counting some thin twine

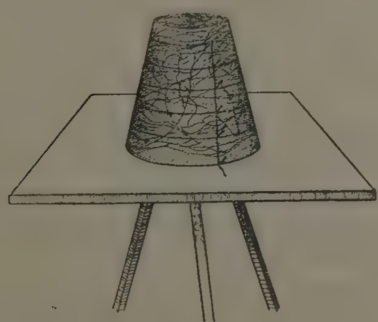

Diagram to show method of cyst estimation on the roots of plants used in pot experiments.

was wound round the circumference of the mass of soil and roots, dividing it into circular bands of about one inch in depth. The major portion of the root system was exposed on the surface of the mass and the white cysts were clearly visible. The few cysts which usually became detached from the roots, adhered to the pot and could easily be counted.

Cyst counts carried out in this manner on the potato plants grown with and without mustard are given in the following table (p. 84).

In comparing the average of the counts on the plants grown with mustard with the count obtained from the control, the reduction in

number of cysts on the former was seen to be 842, or 66·9 per cent. of the count on the control.

This result, which confirmed the previous observations on plants grown with mustard, appeared to be of some significance as indicating a possible means of control adaptable to field conditions. It was not, however, apparent by what means the reduction in the number of cysts was effected. Therefore, before field experiments other than of a purely empirical nature could be carried out, the nature of the factor inhibitory to cyst formation which was introduced into the soil by growing mustard

	Pot 1. With mustard.	*Pot 2.* With mustard.	Average count of *Pots 1 & 2.*	*Pot 3.* Control without mustard.
Total No. of Cysts ...	534	299	416	1,258
No. of Cysts per sq. inch	4·53	2·45	3·49	9·32

seedlings remained to be discovered. With regard to the possible methods by which the reduction might be effected, the following points seemed worthy of investigation.

1. Was the reduction merely due to an increase in the organic matter present in the soil, produced by the addition of the mustard seedlings ?

2. Was the reduction due to some chemical substance present in the mustard seedlings and liberated into the soil by their decay : if so, in what part of the plant did it occur ?

3. Was the reduction due to the early hatching out of the larvæ by the stimulus of the growing mustard, the larvæ then dying out in the absence of a suitable host plant before the potato roots had developed ?

4. Assuming the presence of an excretory product given off from the roots of the mustard, did this root-excretion inhibit the hatching out of the larvæ ?

5. Again assuming that root-excretions are given out by growing plants, did the mustard root-excretion neutralise the potato root-excretion in such a way that (*a*) the larvæ did not receive the stimulus necessary to hatch from the cyst, or that (*b*) the chemiotactic action of

the latter, which might normally cause the larvæ to find the potato roots, ceased to function ?

Dr. Morgan set up a number of experiments in an attempt to elucidate some of these points. Potato sets were planted in pots containing whole mustard plants, shoots only, roots only, a few grains of culinary mustard and soil in which mustard seedlings had been grown and then removed. Pots containing potato sets in infected soil only were set up as controls. Unfortunately when these pots were examined the cysts were found to have turned brown. It was, therefore, extremely difficult for an even comparatively accurate count to be made, and the results were, therefore, discarded as unreliable.

Experiments to test the effect of organic manures were also set up in pots containing the following compounds :—

Pot 1 contained soil to which a synthetic manure called " Adco " had been added.

Pot 2 contained soil to which a balanced dressing of artificial manures was added.

The deficiency of " Adco " in potash and phosphates was made up with artificial manures so that both pots should receive the same quantities of manurial ingredients. These pots were planted and examined on the same dates as the previous experiments, and again the same difficulty was met with in counting the cysts. There seemed, however, to be a greater number present on the roots of the plant in *Pot 1*.

Some work was also commenced in the laboratory to determine factors which might influence the hatching out of the larvæ from the cysts. Twelve cysts were placed in each of nine glass wells and the following liquids were added :—

1. Distilled water.
2. Tap water.
3. Potato root-extract (cold extraction).
4. Potato root-extract (hot extraction).
5. Soil extract from infected Lincolnshire soil.
6. Soil extract from soil in which a potato plant was at the time growing.
7. Extract from sterilised soil.
8. Soil extract and cold root-extract in equal parts.
9. Extract from Hertfordshire soil.

The cold root-extract was obtained by adding 30 ccs. of distilled water to 3·45 gms. of wet potato roots which had been crushed with a pestle. The hot root-extract was obtained by using the same proportions of distilled water and potato-root which was then brought to boiling point and allowed to cool. The soil extracts were obtained by adding 10 ccs. of distilled water to 2 gms. of soil. This was allowed to stand for three hours and was then centrifuged, filtered, and diluted to a quarter of its strength. The object of this experiment was to discover whether an excretion from the roots was a factor influencing the hatching out of the larvæ, or whether moisture alone was sufficient. The wells were kept at room temperature but the experiments gave no definite results, their failure being probably due to lack of warmth and the small number of cysts used.

From this point the investigations were taken over by the present writer.

Methods Adopted in Subsequent Work.

The lines on which the research has been carried out have been indicated in the list given above of points requiring investigation. These questions have been dealt with separately and much experimental work has been carried out to elucidate them and the further problems which have arisen during the course of the work. Some of these experiments are described and the conclusions they lead to discussed in this paper.

Pot experiments similar to those already described have been largely used in the early work and Dr. Morgan's methods of cyst extraction from soil and cyst estimation on the roots of experimental plants have been adopted. The high percentage of probable error which necessarily occurs throughout the work has been taken into consideration in discussing the results.

Preliminary Experiments.

It was thought advisable as a preliminary measure to repeat yet again the experiments conducted by Dr. Morgan, to further confirm the inhibitory influence of mustard on cyst formation. An exactly similar experiment was therefore performed in 1927 and was again repeated in 1928.

Mustard seedlings were grown in Lincolnshire soil to a height of about three inches. These were then broken into the soil and potato sets were planted. Control pots of potatoes without mustard were planted on the same dates. After forty-one and eighty-five days, respectively the pots were turned out and cyst counts were made. In 1927 the count on the experimental plant (mustard) was 866 cysts as compared with 2,278 cysts on the control, and in 1928 the experimental plant (mustard) bore 249 cysts and the average count of two controls was 1,491. The reduction in the number of cysts on the roots of the plants treated with mustard was as compared with the number of cysts present on the control plants 61·9 per cent., in the first case and 83·3 per cent. in the second. That is, the average reduction was 72·6 per cent., a slightly higher figure than that obtained by Dr. Morgan.

The significance of these experiments beyond the fact that they confirm the original observations on the effect of mustard, will be discussed later together with other results.

Effects of Increase in the Organic Content of the Soil.

Since the mustard seedlings broken into the soil had, in these experiments considerably increased the normal organic content of the soil in which the experimental plants were grown, and no corresponding change had been made in the composition of the soil used for the control plants, the reduction in the number of cysts developed in the former case might conceivably be due to this cause alone.

In order to test whether the reduction was due to this cause or to some property inherent in the mustard plant, the following experiments were performed. Five 6-inch plant pots containing equal quantities of infected Lincolnshire soil were prepared. Mustard seedlings were grown in one pot to a height of three inches. They were then removed, washed, weighed and broken up, and returned to the pot. An equal quantity of Adco was mixed with the soil in the second pot, and the same quantity of grass and leaf mould was added to the third. The other two, which contained infected soil only were used as controls. A potato was planted in each of these pots on the same date and allowed to grow for eighty-four days. Cyst counts were then made, the results of which are shown in the following table (p. 88).

The disparity between the cyst count in *Pot A* treated with Adco, and the average of the controls, while being sufficiently slight to be attributable to the margin of error, might have been due to the inclusion in the Adco of a small quantity of the same or some similar substance to that present in the mustard seedlings which inhibited the formation of cysts on the plant in *Pot C*. That some such inhibitory factor is present in mustard is amply demonstrated by the results of this series of experiments.

Pot A Adco.	*Pot B* grass and leaf-mould.	Average of *Pots A and B*.	*Pot C* mustard.	*Pot D* control.	*Pot E* control.	Average control.
1,244	2,125	1,684	249	1,665	1,317	1,491

Effects of Various Portions of Mustard Seedlings.

In order to determine whether the reduction in nematode attack was due to the presence of some chemical substance in the mustard seedlings, and, if so, in what part of the plant it occurred, another series of experiments was performed. This series, first carried out in 1927 was repeated in 1928 when it included the mustard experiment and the controls cited above. The experimental pots were set up as follows :—

Pot 1. One hundred mustard seedlings were grown in infected soil to a height of three inches. They were then broken into the soil in which they were grown and a potato set was planted.

Pot 2. One hundred mustard seedlings were grown in clean soil to a height of three inches. They were then sieved out, washed and broken up in infected soil in which a potato was planted.

Pot 3. Of mustard seedlings grown in clean soil a number of shoots equal in weight to one hundred whole seedlings used in *Pot 2*, were broken into infected soil in which a potato was then planted.

Pot 4. Of mustard seedlings grown in clean soil, a quantity of roots equal in weight to one hundred whole seedlings were washed and broken into infected soil in which a potato set was planted.

Pots 5 and *6*. Potato sets in infected soil without mustard were grown as controls.

The results of the cyst counts made on the roots of these plants and the percentage of reduction occurring in the experiments is shown in the following table.

Before making an analysis of these results further stress should be laid on the high probability of error introduced by the technique. The soil used was from a heavily infected Lincolnshire field and was thoroughly mixed in a bin before being apportioned into pots. The cyst content was therefore approximately even throughout but slight variations must undoubtedly have existed. The plants were grown under identical conditions in a cool greenhouse, and no marked difference was noted between them as regards either shoot or root development, but the inevitable slight variation in the latter must have influenced the cyst count. Finally the method adopted for the estimation of the cysts in itself introduces a wide margin of error.

		No. of days grown.	No. of cysts on roots.	Average No. of cysts on control.	Per cent. reduction.	Average reduction.
Pot 1.	1927	41	866	2,278	61·9	72·6
	1928	85	249	1,491	83·3	
Pot 2.	1927	41	452	2,278	81·1	60·2
	1928	85	889	1,491	40·3	
Pot 3.	1927	41	1,549	2,278	32·0	24·6
	1928	85	1,233	1,491	17·3	
Pot 4.	1927	41	627	2,278	72·4	69·2
	1928	85	504	1,491	66·1	

Notwithstanding these difficulties, the similarity in the counts obtained on two successive years in these experiments assume some significance as indicating the effect on nematode attack of the various portions of the mustard plant.

Pot 1 contained whole mustard seedlings, and, in addition any excretory products which might be given off from the roots of the growing mustard seedlings since the seedlings were broken up in the soil in which they had been grown. The greatest reduction in cyst formation occurred in this

experiment. *Pots 2, 3* and *4* contained whole plants, shoots, and roots respectively but none of these having contained the growing seedlings could be influenced by excretory products of the growing roots. *Pot 3* which contained shoots only, showed only a slight reduction in the cyst count as compared with the controls. *Pot 2*, which contained shoots and roots showed a higher reduction, while *Pot 4*, which contained roots only, showed almost as great a reduction as *Pot 1*.

Since the quantity of material broken into the soil was uniform in each case these results lead to the following conclusions. As the mustard shoots alone when broken into the soil are sufficient to cause some reduction in the cyst count, and as this reduction is not entirely due to the increase in organic matter present in the soil, as shown by a previous experiment, it may be assumed that some substance present in the mustard shoot is liberated into the soil and produces this effect. Where the quantity of mustard broken in is composed partly of roots and partly of shoots, this inhibitory influence is more marked, and where roots entirely replace the shoots it becomes yet more conspicuous, in fact, the reduction is almost three times as great as when shoots alone are used. This chemical substance then, while present in all parts of the plant, is either especially effective by being more readily liberated, or is present in greatest quantity in the roots.

In comparing the results of *Experiment 1*, with the results of *Experiments 2, 3* and *4*, a further factor presents itself. Except that the mustard seedlings were grown in the same soil in which the potato was planted this experiment was exactly similar to *Experiment 2*. The average reduction in the number of cysts is however, greater, and further, it exceeds the reduction in *Experiment 4* although the proportion of roots used was in the latter much greater than in the former case. This suggests that, in addition to the inhibitory substance being present in the plant, it is given off from the roots of the mustard seedlings during growth and accumulates in the soil. Since the mustard was allowed to grow for twenty-three and thirty-nine days respectively in 1927 and 1928 before the potato was planted, and a further period ensued before root development of the potato could take place to any considerable extent, it follows that this substance given off into the soil must retain its active principal for a considerable length of time. The fact that the reduction in the number of cysts was more marked in *Experiment 1* when

the mustard was allowed to grow for thirty-nine days before being broken down (1928) than on the first occasion when it was broken down after twenty-three days, supports this suggestion.

Effects of the Proximity of Growing Mustard Roots on Cyst Formation.

In order to test whether growing mustard roots in close proximity with the roots of the potato in infected soil gave any protection against nematode attack, and thus to confirm the hypothesis of root-excretion, another experiment was performed. Mustard seeds were sown in two pots of infected soil in which potato sets were planted at the same time. The control pots used in the series of experiments described above for 1928 were planted on the same date and served as a control for this experiment also. In the experimental pots the mustard was allowed to grow with the potato plant throughout, the tops being cut after about six weeks to prevent flowering. Cyst counts made eighty-five days after the pots were planted, showed 6 cysts and 159 cysts on the roots of the potato plants grown with mustard, and 1,665 cysts and 1,317 cysts on the roots of the control plants. That is, the reduction in the number of cysts on the experimental plants was 99·5 per cent. and 89·4 per cent., respectively, of the average control count. The average reduction was 94·3 per cent.

While quoting this very high figure it should be noted that the general development of both shoot and root systems of these two plants grown with mustard were very markedly inferior to the development of the control plants. Thus the comparison is by no means an accurate one and must give a disproportionate idea of the comparative intensity of attack Nevertheless the root systems of the experimental plants were strikingly free from cysts as compared with the controls. The poor development noted in these two plants was no doubt the result of overcrowding and had been anticipated when the experiments were set up. Later work has been carried out in which this difficulty has been overcome and this will form the subject of a later publication.

One more experiment was conducted in connection with these investigations which should be recorded here. In order further to prove the influence of growing mustard roots on the infectivity of *H. schachtii*, different quantities of growing mustard were tested. Two hundred mustard seedlings were grown in one pot of infected soil (*Pot A*) and

twenty-five in another (*Pot B*). After thirty-nine days they were sieved from the soil, those from *Pot B* discarded and those from *Pot A* divided into two equal parts, one part being broken up into each of the *Pots A and B*. Potato sets were then planted and a control was set up. After eighty-five days cyst counts were made with the following results :—

Pot A, which had contained 200 growing seedlings showed 906 cysts. *Pot B*, which had contained twenty-five growing seedlings showed 1,317 cysts. The count on the control plant was 1,809 cysts. Thus *Pot A* showed a reduction of 49·9 per cent., and *Pot B* a reduction of 27·1 per cent. Since both pots contained 100 broken up seedlings the difference between the counts must be attributed to the influence of the extra 175 seedlings grown in *Pot A*. By ignoring the margin of error, the inhibitory influence exerted by 175 seedlings growing for thirty-nine days may be estimated as resulting in a reduction of 22·8 per cent. of the normal cyst count.

Conclusions.

The first experiments conducted by Dr. Morgan with mustard, indicate that these seedlings grown and broken up into soil heavily infected with *Heterodera schachtii*, effectively limit the nematode infestation on a potato plant immediately thereafter grown in that soil. This conclusion has been amply confirmed by subsequent experiments.

That the reduction in nematode attack is not solely due to the increased organic content of the soil has been shown by substituting other organic matter for the mustard seedlings. Some chemical constituent of the mustard plant, not normally present in soil, must therefore be released by the decomposing plants, and in some manner not so far demonstrated, check the nematode at some period of its life history.

Experiments have proved that this substance is present throughout the plant. It is, however, either present in greater quantity or is more easily liberated from the root system than from the shoots. Further, there is evidence that the growing roots exercise a stronger influence in diminishing the infectivity of the nematode to the potato plant than do the broken up roots when mixed with the soil. It is therefore concluded that the substance inhibitory to the nematode, besides being present throughout the plant is given off by the growing roots.

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Observations on the incidence of *Heterodera schachtii* at the Ormskirk Potato Testing Station.

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Introduction.

The Potato Testing Station at Ormskirk, a branch of the National Institute of Agricultural Botany, has during the past season, been found to include considerable areas of land heavily infested with the eelworm *Heterodera schachtii*. This discovery was made subsequent to the failure of some of the potato crops, which, in the course of the normal rotation practised at the station, happened last season to be grown on the affected areas.

The occurrence of the nematode having been reported to the Institute of Agricultural Parasitology, a survey of some of the land belonging to the station was carried out, to determine the extent and severity of the infection.

Methods of Investigation and Results of Estimation of Cysts in Soil.

These areas were systematically examined, and estimations of the "brown" cyst content of the soil were made. Samples of surface soil were removed at distances of ten to twenty yards apart, the positions from which they were taken being noted. The cysts were then extracted by Morgan's flotation method and the number present in each sample was roughly estimated. In addition, measured samples of five cubic inches of soil from each of the plots under investigation were also taken and

the number of cysts extracted were accurately counted to determine the proportional infection present in the soil. The areas thus examined were chosen by the Staff of the Station, whose observations during the period of growth have been of great assistance.

Plot A (Field 18, east or lower end) had proved unsatisfactory in the 1928 season, the plants showing marked signs of disease and being heavily parasitised by the eelworm. *Rhizoctonia* was also present on the plants grown on this area.

Plot B (Field 18, west or top end) had produced good crops, the plants being free from signs of disease, but cysts were present in fair numbers on the roots.

Plot C (Field 15) representing about four acres of land, had been under rotation for about nine years but was to be used for potatoes in 1929.

The cyst content of *Plot A* was found to be very heavy and uniform throughout, 125 cysts being extracted from an average sample of five cubic inches. In *Plot B* the cyst content was found to vary, being moderately heavy in some areas, slighter in others. This suggests that the eelworm, having been introduced in isolated patches, has been spread, possibly to some extent by the processes of cultivation during the years of rotation, and is gradually becoming established over the whole area. The average cyst content of this soil was found to be twenty-eight cysts to five cubic inches. *Plot C*, as stated above had been under rotation for a number of years. The 1928 crop had been a cereal, but a small number of potato plants were found which had evidently sprung from overlooked tubers of the last potato crop, which had increased in number through the succeeding years. This land was found to be lightly but uniformly infected throughout, except in the immediate vicinity of the growing potato plants where the cysts were rather more numerous. Fifteen cysts was the average content of five cubic inches of soil.

Conclusions Based on the Cyst Content of the Soil.

In comparing the cyst content of the soil from the three plots, it should be noted that *Plot B*, in which the potatoes were unaffected in 1928 contained 22 per cent. only of the infection present in *Plot A* where the crops were a failure. But whereas in *Plot A*, *Rhizoctonia* was present in most if not all of the plants, it had not been observed in *Plot B*.

Plot C contained only 12 per cent. of the infection present in *Plot A*,

and 55 per cent. of the infection present in *Plot B*. Since the 1928 crop on *Plot B* remained unaffected by the presence of the parasite, the 1929 crop on *Plot C* should not be reduced by the attack of the eelworm present in this land, unless some complicating factor intervenes.

Experiments on the effect of *H. schachtii* alone and *H. schachtii* in conjunction with *Rhizoctonia* are now being performed at the Institute's Field Station, Winches Farm.

Observations on the Morphology of the Cysts.

The cysts extracted from *Plots A* and *B* were large and of the usual rounded type associated with *H. schachtii* on the Potato. From 30 per cent. to 40 per cent. had the appearance of being newly formed, that is, they were light in colour, entire and unwrinkled, and were found on microscopical examination to contain numerous eggs at an early stage of development and very few shells from which the larvæ had escaped.

An interesting feature of the infection in *Plot C* was that, although so long a rotation had been adopted, many of the cysts extracted from this soil, even where no potato plants were growing, also had the appearance of being newly formed. Further, over 60 per cent. of the cysts, instead of exhibiting the rounded form typical of the cysts formed on the roots of the potato were more elongated and lemon-shaped, with the vulva distinctly visible at the posterior end of the body. These lemon-shaped cysts were in every case small, usually not more than half the size of the rounded cysts present in the same soil.

Cysts extracted from a quantity of this soil were mixed with clean loam in which potato, mangold and beet seeds were planted. Four months later the roots of the seedlings were examined, and those of the potato seedlings were found to bear a considerable number of white cysts, and some which had already turned brown. The mangold and beet seedlings were not attacked.

The brown cysts formed on the potato roots were, without exception, of the rounded type. The white cysts varied in shape according to their size. The larger ones, which were, presumably, fully formed, were, like the brown cysts, approximately spherical; the smallest were distinctly ovoid, their width representing from three-fifths to three-quarters of their length. Cysts of intermediate size were intermediate in form between the ovoid and the spherical. In no case was the vulva visible

as a protusion at the posterior end, as was the case in the majority of the cysts with which the infection was produced.

Goffart, however, has shown in a recently published paper, that the rounded shape of the cysts of *H. schachtii* on potatoes is merely a host reaction, and that, when a strain of *H. schachtii* from potatoes can be induced to attack sugar beet, the new cysts formed on the beet assume the lemon-shaped form typical of a natural infection on that host. The present author has shown that an unfavourable environment such as heavy soil has the effect of greatly reducing the average size of the cyst. Further, recent observations have shown that in some cases where *H. schachtii* is present in the soil in this country a number of weeds, including several common grasses may be attacked.

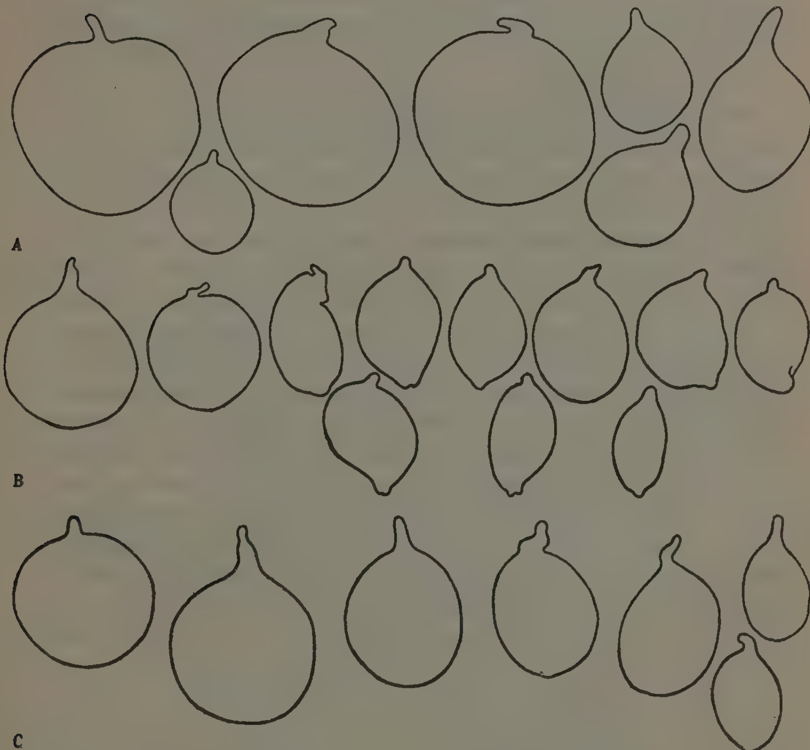
In view of these observations, the newness, shape and size of the cysts in this Plot assume some significance. It seems possible that the parasite, lacking the usual host plant, may have succeeded in parasitising to a small extent, either some crop used in the rotation, or, more probably, one or more species of weed which occur constantly upon the land.

Experiments to Determine Whether Cysts Remain Viable After Passage Through the Pig.

In previous years it has been the custom for the potato crops produced by the Testing Station to be used as pig-food. Since, however, a small quantity of soil must inevitably remain adherent to the tubers, and, in addition, white cysts had been observed actually attached to the tubers when freshly lifted, it was thought that, by feeding these tubers in an uncooked condition to pigs, a small quantity of cysts must necessarily be ingested. If then, the parasite remained viable after passage through the pig, the pig-manure when spread upon clean land would prove a fertile source of infection. In order to determine whether the eggs and larvæ of ingested cysts retain their viability the following experiments have been performed.

A large number of brown cysts were extracted from soil by Morgan's flotation method. These were mixed in the original experiment with milk, and in a repetition experiment with "sharps," and fed to two young pigs which had previously been kept without food for twelve hours. The fæces of the pigs were then collected at intervals and examined for cysts.

After a period of twelve hours numerous cysts appeared in the fæces, and they continued to be passed in gradually diminishing numbers for a period of forty-eight hours. The cysts were again extracted from the fæces, and samples of fifty cysts passed at intervals of twelve, eighteen,



A.—Cysts extracted from soil of *Plot A* after removal of potato crop, 1928. (× 33.)
B.—Cysts extracted from soil of *Plot C* after nine years of rotation crops. (× 33.)
C.—Cysts from roots of potato seedlings experimentally infected by cysts from *Plot C*. (× 33.)

twenty-four and thirty-six hours after ingestion were kept in petri-dishes at a temperature of 20° C., in water in which potato-seedlings had grown.

As a control measure cysts were isolated from soil and kept under observation under similar conditions. The result of these experiments has been that in no case did any larvæ emerge from the cysts that had passed through the pigs, though after a period of seven days the larvæ in the control experiments were hatching freely and in numbers. Microscopic examination of the contents of the former cysts showed that the larvæ that were free within the cysts were dead and already showing signs of decomposition while those enclosed within the eggs, when liberated by artificial means were also found to be dead.

Baermann extractions were made of the freshly passed fæces to determine whether larvæ were liberated within the alimentary canal of the pig, but by this means very few larvæ were extracted and these again proved to be dead.

The remainder of the cysts passed by the pigs were mixed with soil in which potatoes were planted. Four months later the roots of these plants were examined and were found to be free from cysts.

The conclusion based on these experiments is that the larvæ and eggs in the cysts of *H. schachtii* do not remain viable after passing through the pig. In this connection, however, it should be noted that the animals used were only about six weeks old, and that therefore the cysts were subjected to a slightly higher temperature than would have been the case had adult animals been used. In view of Fuchs's observations on the effect of heat on the cysts of *H. schachtii* from sugar-beet it would appear that temperature is not the decisive factor in this case, and a further series of experiments to determine the effects of various digestive secretions on the cysts will shortly be carried out.

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On Fairley's Intradermal Reaction in Schistosomiasis.

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In 1927 Fairley described in the *Medical Journal of Australia* an intradermal reaction in human Schistosomiasis, using as antigen the dried powdered livers of snails (*Planorbis exustus*), infested with the cercaria of *Schistosoma spindalis* of the Goat, dissolved in saline. The standard dose adopted was 0·25 cc. of an 0·5 per cent. extract prepared from the dried powder, ground up in a mortar with 50 ccs. of normal saline solution (0·85%) and the resulting mixture thoroughly shaken by hand. It was then incubated for one hour at 37° C. Subsequently the extract was centrifuged, the supernatant fluid pipetted off and filtered through a Seitz filter (size 3) attached to a water pump. The filtrate, consisting of a clear coloured fluid is put up in ampoules of 0·5 cc. each and stored in an ice-chest.

When injected intradermally the reaction was described as the appearance of a large white wheal, 2 to 3 cm. in diameter with pseudopodia-like out runners and a surrounding zone of erythema. Seven out of eight patients who had been suffering from *Schistosoma hæmatobium* infections gave immediate reactions.

Fairley considers the test to be exclusively of diagnostic value and as not affording any index of the effect of drug treatment. Reactions were given by patients who were regarded as cured on clinical grounds.

Early in February of this year, and within two days of one another, I was able to diagnose a heavy infection with *Schistosoma hæmatobium* in two young men aged 25 and 28 respectively who had contracted the infection whilst bathing in the Transvaal or in Natal. They both received treatment by intravenous injection of Sodium Antimony Tartrate simultaneously. By March 15th, 1929, Case A had received

22 grains intravenously and Case B 21 grains. The urine had completely cleared and after the injection of 19 grains in either case no eggs could be demonstrated. Five days subsequent to the cessation of treatment Fairley's intradermal test was given. The antigen was prepared by Fairley himself according to the formula given above. In each case 4 minims of a saline extract of the powdered liver of *Planorbis exustus* infected with cercariæ of *S. spindalis*, and which had been stored since the 8th of November, 1928, was administered. It is to be noted too for future reference that the antigenic power of the extract is particularly stable for the livers themselves were dried and powdered some three years previously, in 1925.

An area of skin on the forearm was shaved and the antigen was injected intradermally by means of a fine hypodermic needle. As a control, a similar injection was given to myself. In the latter instance, beyond a wheal at the site of injection, nothing resulted. In the two patients, however, a most marked reaction commenced (see Plate) within ten minutes of the injection. A raised whitish and glistening wheal appeared at the site of injection and running out from it were two or three pseudopod-like out runners. There soon appeared an angry red and irritating ærola. The wheal itself in each case was 2 cm. in diameter, the surrounding inflammatory reaction about 30 cm.

The reaction in each case remained visible for 24 hours before it finally faded. In one case after thirty hours there was a secondary delayed reaction as described by Fairley. Both men described the skin reaction as causing irritation and discomfort.

The figure which accompanies this article shows the type of skin reaction which is given by this test. It is so immediate and so striking that this particular intradermal test is assured of a definite rôle in the diagnosis of Schistosomiasis.

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P.M.-B. *del.*

Schistosoma hæmatobium infection of two years duration. Fairley's intradermal test on skin of forearm showing central wheal with outrunner and surrounding erythema.
Natural size. Painting made ten minutes after injection.

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Landmarks in Medical Helminthology*

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May I say, in the first place, how highly honoured I am by the invitation of the Royal Faculty of Physicians and Surgeons of the City in which I received my early medical training to give the sixth Finlayson Memorial Lecture.

It was not my good fortune as an undergraduate to become a member of Dr. Finlayson's Clinic. As far as my recollection goes I saw him on one occasion. It was a chance encounter, in a corridor of the Western Infirmary 25 years ago, as he was about to make his daily visitation. In that fleeting moment I caught and have retained a clear impression of his kindly face and that recollection gives me a special pleasure to-day. Dr. Finlayson was a wise and skilful physician, but he was profoundly learned in medical archæology. The library of the Faculty owes much to his enthusiasm as honorary librarian during a period of service extending over a quarter of a century. The bibliographical demonstrations of rare and old books given by him in this Faculty Hall were notable events in his day. I hope, therefore, that it may appear appropriate that I have chosen the historical aspect of helminthology as the subject of this Memorial Lecture.

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* A Lecture delivered before the Faculty of Physicians and Surgeons of Glasgow, on February 21st, 1929.

Helminthology has recently been described as a branch of Zoology.

According to the most recent computation the number of helminth species which occur in the human body is one hundred and nineteen, of which thirty-one are important either on account of their wide prevalence or association with disease.

The word Helminthology, however, has no exact meaning in Zoological classification, whereas the term can be used at the present day as a phrase of medical terminology in the same sense as it was used by SIR WILLIAM RAMESEY, Physician to His Majesty King Charles the Second, when he published, in 1668, what would appear to be the first English Text Book on this subject. The volume is entitled " Elminthologia, or Some Physical Considerations of the Matter, Origination, and Several Species of Wormes Macerating and Direfully Cruciating every part of the Bodies of Mankind, etc." It deals not only with the parasites and their origin, but also with their distribution in the body; the susceptibility of the various ages of man; the factors rendering man liable to them; the divers signs whereby they may be discovered; the symptoms and prognosis; and lastly, the methods of cure " with a cautionary direction how to prevent and remedy those direful evils by them occasioned."

The number of species then generally recognised was stated by RAMESEY to be three, viz., large maw-worms, Longi and teretes, tapeworms, Lati lumbrici, and (female) seat-worms then known as Ascarides. All these forms had been collectively named "Helminthes" by HIPPOCRATES and had been known from very early times.

Probably this early knowledge of their presence in men arose not so much from their size as from the fact that, under certain and often distressing circumstances, they make their way out of the human body. That the Greeks and Romans were so well acquainted with the minute seat-worm is undoubtedly attributable to the anal irritation which its customary migrations caused. Until RAMESEY'S time worm infestations had been regarded not as the cause, but as a product of disease, and as arising " from putrid, vitious and gross, viscid, corrupt matter of what nature so ever, having a vital principle in itself apt for generation." " What I aim at in this Essay," he wrote, " is to put both Physitian and Patient in a Jealousie, on any Distemper, that worms are the

original cause, especially in those that have any thing of Fermentation and Putrefaction." And in an italicised paragraph he makes, for that time, the profound suggestion: "They may have their origination in us by contagion, from certain animated effluviums or vermicular atome-like corpuscules or Ferments which flow out of gross, corrupted bodies and fly through the air, whereby they are communicated to bodies capable of and fitted to receive such impressions; and so by their evil and venemous ferment, are infected; which many times, so lurk in the blood and humours that they occasion strange diseases and symptoms."

A fourth species, which is not mentioned by RAMESEY, but which was known to the ancients and of which accounts had appeared in the reports of travellers in tropical lands, is the guinea-worm, which on eruption on the limbs caused painful ulcers and abcesses. PIGAFETTA, in the second part of the account of his travels, gave in 1598 an interesting plate showing a native extracting a guinea-worm from his own leg. In the background one sees also a surgical operation proceeding upon the eye of another patient. This has been held to be the first record of the eye-worm Loa.

VELSCH, in 1674, published a large monograph describing the guinea-worm and discussing the opposing theories as to its nature. In 1683, DR. TYSON communicated a paper to the Philosophical Transactions of the Royal Society entitled "Lumbricus teres, or Some Anatomical Observations on the Roundworm Bred in Human Bodies." The plate accompanying this paper gives an extraordinarily exact representation of the internal anatomy of the male and female *Ascaris lumbricoides*. In the same year he beautifully illustrated the naked eye appearances of a tape-worm which he calls *Lumbricus latus*. The head of the specimen was missing, however. A complete tape-worm showing a large black head was found in 1669 and illustrated by DR. ANDRY of the Faculty of Medicine, Paris, in his monograph "De la Génération des Vers dans le Corps de L'homme," published in Amsterdam in 1701. While examining segments of this worm under the microscope Andry observed minute round bodies which he recognised as ova. This discovery led him to state that the eggs of worms enter our body with air and food. In the chapter dealing with treatment, Santonin, Male Fern and Pomegranate are stated to be good for worms. With the publication in 1758 of LINNÆUS' "Systema Naturæ" the parasitic worms

of man received formal names: *Tænia solium*, *Tænia lata*, *Ascaris lumbricoides*, *Ascaris vermicularis*, and *Gordius medinensis*. In 1762, the seat-worm was made the subject of an extensive monograph by VAN PHELSUM and this was accompanied by figures of considerable accuracy.

An outbreak of mucous fever in the city of Göttingen in 1760 led to the discovery of the whip-worm by ROEDERER. This was described in the following year under the name Trichuris given in the belief that the thin whip-like portion of the worm was the tail.

Specimens of the liver fluke of sheep were found in human cases by PALLAS (? 1768) and BUCHHOLZ reported great numbers in the gall bladder of a slave who had died of putrid fever.

GUYOT, a French Ship's Doctor who had visited the west coast of Africa, published a short note in 1778 stating that he had seen the natives removing from the eyes worms which were called "Loa" locally.

In 1780 a scientific society was founded in Copenhagen to investigate the nature and habits of Hydatids, and two years later GŒZE succeeded in showing that the Hydatid cysts possessed hooks and suckers and were related to the Tæniæ. BATSCH in 1786 formally named the Hydatid "*Hydatigena granulosa*." Another important contribution made by GŒZE was the differentiation of the Tænias of man hitherto known under the name "solium" into two distinct varieties which he named Tænia cucurbitina, grandis, saginata and Tænia cucurbitina, plana, pellucida.

That the muscles of pigs are sometimes measly or infected with bladder worms had been known from very early times. In 1276 it was laid down in the city of Augsburg that "if a butcher kill a measly hog he shall sell it to no one without a statement of this fact." Bamberg, Zwickau and various other German city states had prohibited the sale of measly meat during the fourteenth century. It was not until 1786 that a case in man was described and illustrated. WERNER found a large number of specimens in a body prepared for an anatomical lecture. Seven years later TREUTLER illustrated and described a case of invasion of the brain. In the same communication this author described a new nematode, *Hamularia lymphatica*, from the bronchial lymphatic glands and two other new parasites which have remained unidentifiable to the present day.

The last years of the eighteenth century and the opening years of the nineteenth were remarkable for tremendous and sustained interest in the parasites of all kinds of animals. The interest, however, lay chiefly in their zoological description and classification.

PALLAS in 1781 had attempted unsuccessfully to transmit parasitic worms by feeding eggs to dogs, and the old idea attributing the origin of helminths to spontaneous generation had become almost universally adopted. RUDOLPHI, whose careful systematic work from 1793 to 1819 brought him such well-deserved prestige that he became known during the succeeding century as the Father of Helminthology, was a strong supporter of the doctrine of spontaneous generation. In his monograph of 1808 he refers to the discovery of minute tape-worms in vast numbers in the intestine of the dog. From their small size and close attachment to the intestinal mucosa he believed that they were the young of *Tænia cucumerina* which were arising from tags of intestinal mucosa by spontaneous generation. It was not until forty years later that their independent character was recognised and their relation to the Hydatids of man and animals surmised.

1819 brought DR. BREMSER'S monograph "Ueber Lebende Würmer im Lebenden Menschen." This volume lists twelve helminth parasites of man, including two of TREUTLER'S species. The plates which illustrate the text show that while careful attention had been devoted to external characters these had been depicted with the aid only of the simple lens and that the compound microscope had not yet come to service for the elucidation of internal structure. Nothing was yet known of the development of any of the parasites of man, but DR. BREMSER makes a notable addition to medical helminthology by describing and illustrating for the first time the male of the seat-worm *Oxyuris vermicularis* specimens of which had been passed after an oil clyster administered by the famous SOMMERING to his son. RUDOLPHI'S "Entozoorum Synopsis" also appeared during 1819 and with its publication scientific activity ceased for a period of years.

During the remainder of the first half of the eighteenth century only four discoveries call for comment. In 1835, the anatomist OWEN gave the name *Trichina spiralis* to certain tiny encysted worms which MR. WORMALD of St. Bartholomew's Hospital had found in the muscles of an Italian who had died in London of tuberculosis. The clinical

significance of Trichinosis was not appreciated until thirty years later. OWEN expressed the view that "the number of individuals infesting the body was so immense, and their distribution throughout the muscular system so extensive, that they might occasion debility from the quantity of nutriment required for their support." He added, however, that "no painful or inconvenient symptoms were present in any of the cases and it is probable that in all cases the patient himself will be unconscious of the presence of the microscopic parasites which are enjoying their vitality at his expense."

While dissecting the body of a peasant woman, who had died in his hospital at Milan in 1838, ANGELO DUBINI came across a small round intestinal worm of an unusual kind. But it was not until 1843 that he was led by a second discovery to publish his observation. The great hygienic significance of this worm, *Ancylostoma duodenale*, does not appear to have been recognised at this time although in the text book "Entozoografia umana" published by DUBINI in 1850 he notes that in some cases the number of these worms was so great as to justify him in ascribing death to their presence.

At this period two workers in America made contributions of scientific interest. In 1842, WEINLAND described as a new species *Tænia flavopunctata*, a tapeworm which had been found by DR. PALMER in a child in Boston, U.S.A. This worm has since been shown, however, to belong to *Hymenolepis diminuta* of the rat, described by RUDOLPHI in 1819. LEIDY in Philadelphia recorded in 1847 the presence of *Trichina spiralis* in the muscles of pigs.

Finally we must make reference to the publication of two important systematic treatises on the helminths of animals written generally in the tradition of RUDOLPHI, viz., "Histoire Naturelle des Helminthes ou Vers Intestinaux," by DUJARDIN in 1845, and the "Systema Helminthum," published by DIESING in Vienna in 1850.

In illustration of the zoological detachment of these workers we may note in passing DUJARDIN's statement: "On peut se demande si les helminthes sont véritablement nuisibles aux animaux dans lesquel ils habitent. Je suis pour la négative."

Although they had no apparent bearing upon medical helminthology of this period we must briefly recall certain isolated observations in

the zoological world. In 1773, O. F. MÜLLER had described, under the generic name *Cercaria*, a number of independent free-swimming animals. NITZSCH in 1817 had observed that these forms sometimes developed a "pupa" or underwent encystment. In 1818, BOJANUS saw Cercariæ emerging from certain tubular worm-like bodies in fresh-water snails, and in a footnote to Bojanus' paper the editor made the shrewd comment, "one might make a bet that these Cercariæ are the embryos of distomes." In 1831 MEHLIS observed ciliated embryos emerging from trematode eggs, and in 1835 VON SIEBOLD had the remarkable good fortune to find in a trematode parasite of the moor-hen an egg in which the ciliated embryo constantly carried in its interior a cylindrical body similar to those tubular worms from which BOJANUS had already seen cercariæ escape. These isolated facts were linked together in 1842 by the Danish zoologist STEENSTRUP as an additional argument for his theory of an alternation of generations. Later workers brought forward abundant evidence that this explanation was of almost universal application to the life history of distome parasites. But when the theory was enunciated the only distomes which were known to parasitise man were a few cases of accidental infection with the sheep liver-flukes *Fasciola hepatica* and *Dicrocœlium dendriticum*.

With the year 1850 a new era commenced through the introduction of experimental methods in the study of helminthology. HERBST, of Göttingen, successfully transmitted *Trichina spiralis* by feeding experiments. In the following year KÜCHENMEISTER, by similar methods, solved the problem of the transmission of the Tænioid tape-worms. Although the similarity of the heads of bladder worms to those of adult tape-worms had been noted as long ago as 1691 by TYSON, 1782 by GŒZE and 1786 by WERNER, the bladder worms and the tape-worms had been placed by RUDOLPHI and other zoologists in two distinct orders. In 1845, DUJARDIN had written concerning the bladder worms "evidently there is here an abnormal development, a kind of monstrosity, and one might imagine in certain cases that they are the eggs of real Tænias which, conveyed by the circulation into the tissue substance of mannals, have not been able to follow the ordinary developmental phases of their existence." This theory had been adopted by VON SIEBOLD, whose prestige as a zoologist was very great in Germany.

The publication in 1852 by KÜCHENMEISTER, a medical practitioner in Zittau, of the successful results of feeding the bladder worms of rabbits to dogs produced an enormous sensation. From his experiments KÜCHENMEISTER argued that the bladder was a normal but provisional organ for the nourishment of the growing tapeworm, and he maintained that tape-worms had been without exception cystic worms in their youth. He made a careful study of the morphology of the head of *Tænia solium* and of the head found in the bladder worm in the muscles of pigs and arrived at the conclusion that as they were morphologically identical the cystic stage in the pig must be the young of *Tænia solium* of man.

While these important happenings were taking place in Europe a German, DR. BILHARZ, was sending from Egypt, to his former teacher VON SIEBOLD, letters containing accounts of new discoveries in another field. While examining a case which had died of dysenteric symptoms BILHARZ had come upon a new and strange type of fluke which lived in the blood vessels. To this parasite he gave the name *Distomum hæmatobium* in 1852. In the month of April, 1851, he had found large numbers of small tape-worms and of a minute fluke in the intestine of two other cases. These parasites were named by VON SIEBOLD in 1852, *Tænia nana* and *Distomum heterohyes* respectively. BILHARZ described also the peculiar shaped eggs laid in the tissues by the blood flukes and noted that they were of two kinds, some having a terminal spine, others having a spine placed laterally. But all his observations attracted relatively little attention at the time owing possibly to the greater interest of KÜCHENMEISTER'S discoveries.

In 1854 a favourable opportunity presented itself to KÜCHENMEISTER to put to the proof his view that *Cysticercus cellulosæ* of the pig was the larval stage of *Tænia solium*. A woman condemned to be beheaded for assassination was made to swallow three different kinds of cystic worms. The large *Cysticercus tenuicollis* of the sheep was administered one hundred hours before the time fixed for her execution. Twenty hours afterwards she swallowed six specimens of *Cysticercus pisiformis* of the rabbit and at intervals of sixty-four, twenty-four and twelve hours before her death she swallowed fifty-seven *Cysticercus cellulosæ* from the pig. At the post-mortem forty-eight hours after death, ten

young Tænias from four to eight millimetres long were found attached to the intestinal wall and to have all the appearances of young *Tænia solium.* In his account of these experiments KÜCHENMEISTER drew attention to the advisability of an alteration of the police regulations concerning public health so as to provide for some control over the sale of meat infested with Cysticerci.

In the same year (1854) GRIESINGER, writing upon the diseases of Egypt, drew attention for the first time to the *Ancylostoma duodenale* as the direct cause of a severe ailment, Egyptian chlorosis or Egyptian anæmia, which was exceedingly common in Egypt and yet the cause of which up to that time had not been understood.

On the conclusion of his successful experiments with the tape-worm KÜCHENMEISTER turned his attention to the round worm and attempted to prove a relationship between the larval *Trichina spiralis* and the adult worm ; being led to the conclusion from the similarity of their anatomical structure, especially that of the œsophagus. He believed that in LEIDY's discovery of encysted Trichina in the flesh of the pig there was a hint as to how man possibly infected himself with whip-worm. He attempted to infect dogs with whip-worm by administering trichinosed material but these experiments gave negative results. He attributed the spread of infection in *Oxyuris vermicularis* to the migration from one individual to another of female adult worms ; this migration taking place at night between bedfellows. With RICHTER he observed that the eggs of *Ascaris lumbricoides* slowly developed in water to form an embryo, but that this embryo did not hatch and was probably conveyed passively to its new host.

In 1857 specimens of the large intestinal fluke in man, which had been found as long ago as 1843 by MR. BUSK in the duodenum of a lascar who had died in the Seamen's Hospital were named by E. LANKESTER *Distoma buskii* in an appendix to his translation into English of KÜCHENMEISTER's "Manual of Animal and Vegetable Parasites of the Human Body." In December of 1857 DAVAINE published a short communication, "Sur la Diagnostic de la Présence des Vers dans L'Intestin par L'Inspection Microscopique des Matières Expulsées," in which, for the first time the value of routine microscopic examination of fæces for the diagnosis of helminth eggs was recognised. The method had been used by M. DAVAINE to demonstrate the presence of eggs of the whip-worm

and of *Ascaris lumbricoides* in human cases and of the liver-fluke in sheep.

In 1858 BILHARZ's blood flukes were recognised by WEINLAND and by COBBOLD to represent a genus distinct from Distoma which was named Schistosoma by the former and Bilharzia by the latter; the name Schistosoma gaining priority by only a few months over that of Bilharzia.

The *Trichina spiralis* again came to the fore as a subject for research. VIRCHOW was the first to rear and recognise the sexually mature adults in the intestine by experimentation upon a dog. In 1860 LEUCKART published a fine monograph illustrating these forms and describing their structure and development, and in the same year the importance of Trichinosis as a human disease was recognised for the first time as a result of ZENKER's study of a case which had died of typhoid symptoms in the hospital in Dresden. At the autopsy, the muscles were found to contain Trichina embryos which had not yet encysted and in the intestine numerous adult Trichina were found while the characteristic lesions of typhoid were absent. The publication of this case aroused widespread interest and numerous epidemics of Trichinosis were recognised in various parts of Germany.

Meanwhile attention was being directed to the problem of transmission of two other tape-worm infections in man. KÜCHENMEISTER in 1855 had once more drawn attention to the two distinct types of worms which had been so long confused under the name of *Tænia solium*. To the larger opaque form, which lacked the double crown of hooks on the head, he gave the name *Tænia medio-canellata*, limiting the name *Tænia solium* to the more transparent type with armed head and of somewhat smaller size. It is obvious from the illustration that this new species medio-cancellata was that illustrated by ANDRY as the " Ver solitaire " or solium worm, and the specimen named by GŒZE in 1782 as *Tænia cucurbitina, grandis, saginata*. Hitherto, no cystic worm with the characteristic unarmed head had been found in the flesh of food animals. From reports, this unarmed tape-worm was very prevalent among the Abyssinians who ate beef only and preferred it raw. LEUCKART was led to experiment with young calves in order to discover the corresponding bladder worm. These investigations made during November

and December of 1861 proved successful. In 1862 LEUCKART also showed that the Echinococcus hydatid cysts could be produced experimentally by feeding mature segments of the *Tænia echinococcus* from the dog to pig. In the same year NAURYN similarly infected cattle, while in the year following, 1863, the latter worker experimentally infested a dog with adult *Tænia echinococcus* by feeding to it the contents of a hydatid cyst from a human liver. Finally the experiment of infecting pigs with *Cysticercus cellulosæ* by administering the eggs of *Tænia solium* was made by GERLACH in 1869.

In 1863 DEMARQUAY had found nematode embryos in the contents of a chylous hydrocele in a case in Havana, while in 1868 WUCHERER had seen similar embryos in chylous urine in Brazil. LEUCKART, to whom specimens had been sent, considered them to be "embryos of some round worm probably belonging to the Strongylidæ."

In 1869 FEDTSCHENKO in Turkestan confirmed LEUCKART'S hypothesis that the life history of the guinea-worm would resemble that of *Cucullanus elegans*, a parasite of the perch,which he had shown previously to develop in cyclops. FEDTSCHENKO'S paper was published in Russian in 1870 and is very inaccessible. Two illustrations of his work are given by LEUCKART, and it is to be observed that the second figure, which represents the metamorphosed guinea-worm larva, is undoubtedly based on a specimen of a Cucullanus larva.

In 1784 DR. TIMOTHY LEWIS reported the presence of nematode embryos, similar to those previously seen by WUCHERER, to occur not only in chylous urine but in the blood of natives of India. He associated these microscopic filariæ with chyluria, elephantiasis and some allied pathological conditions. In 1876 the adults of these blood embryos were found by BANCROFT in a lymphatic abscess and were named *Filaria bancrofti* by COBBOLD in July of the following year.

Meanwhile, MANSON, then a busy practitioner in Amoy, had noticed that the Filaria embryos in the blood showed a nocturnal periodicity. This he shrewdly associated with the night-biting habits of a common mosquito. And in the China Customs Medical Report for September, 1877, he was able to announce that these filaria embryos underwent further development in the body of *Culex fatigans*. It was not until 1900, however, that the return of the infection to man through the proboscis of the insect was demonstrated by LOW.

In the meantime two new flukes were being described, one, of which at least was to prove later an important parasite in man. Professor McConnel of Calcutta recorded the presence of a large number of flukes obstructing the bile duct in a Chinese, and to this species Cobbold in 1875 gave the name *Distoma sinense*. In the following year Lewis and McConnel described and named *Amphistoma hominis* from specimens collected at a post-mortem on an Assamese by Drs. O'Brien and Curran in 1857. In 1876 a number of French soldiers who had returned from Cochin China were suffering from severe diarrhœa. In their evacuations Dr. Normand discovered a large number of nematodes which Bavay described in 1877 as *Anguillula stercoralis*. Normand, at post-mortems later, found numerous other nematodes in the intestines. These were described as distinct species under the name *Anguillula intestinalis*. It was not until 1882 that Leuckart was able to demonstrate that these two forms were succeeding phases in the development of the same species.

A third Asiatic fluke had come under notice in 1878 when treating a Portuguese from Formosa for symptoms of thoracic aneurism. At the post-morten of the case Dr. Ringer found a small fluke in the lung which he forwarded by Manson to Cobbold, who named it *Distoma ringeri* in 1880. This was the first record of the widespread lung disease Paragonimiasis or Parasitical Hæmoptysis of the Far East.

About this time the making of the St. Gothard Tunnel was accompanied by a marked outbreak of miner's anæmia. Perroncito established, in spite of stubborn opposition on the part of his colleagues, that this tunnel disease was due to hook-worm. In 1881 Bozzolo introduced Thymol to replace male fern in the treatment of resistant cases of hook-worm. It is interesting to note that oil of chenopodium was given a brief test by Baumler and Fribourg but was not considered valuable. In 1882 the life history of *Tænia lata* of the ancients, and *Dibothriocephalus latus* of the present day was partially solved by Max Braun who showed that the larvæ of this tape-worm occurred in certain freshwater fish and could be experimentally transmitted to man and dogs. A larval Bothriocephalus had been found in the tissues of man in 1881 by Manson in China and this was named by Cobbold in 1883 *Ligula mansoni*.

The liver fluke of sheep named by Linnæus in 1758 caused great

ravages in Europe throughout the nineteenth century, but it was not until 1883 that its life history was worked out by THOMAS at Oxford.

In the following year GRASSI and CALANDRUCCIO advised the use of male fern for liver fluke disease. But this valuable suggestion does not seem to have been given much attention. In 1885, BOTKINE described an anæmia associated with Bothriocephalus infection. That hook-worm disease could be acquired through the mouth was experimentally demonstrated by LEICHENSTERN in 1887 and in 1890 CALANDRUCCIO proved that *Anguillula* or *Strongyloides stercoralis* could be similarly acquired.

Two new filaria embryos were found in the blood of African natives by MANSON in 1891, one of which showed a peculiar diurnal periodicity. This filaria diurna has since been recognised as the larval form of the Loa eye-worm. The other embryo to which the name Perstans was given because it persisted without variation through day and night was thought by MANSON in 1892 to be the cause of sleeping sickness. In that year GRASSI and ROVELLI traced the life history of *Tænia flavopunctata* of WEINLAND (1842) through the rat flea. They also showed that the species Murina which is morphologically identical with Bilharz's Egyptian tape-worm *Tænia nana* did not require an intermediate host but formed cysticercoids in the duodenal mucosa of the rat when the eggs were swallowed. Another African filaria worm was brought to light at about this time. Subcutaneous nodules containing masses of filaria worms were sent to LEUCKART in 1893 and were described by him as a new species, *Filaria volvulus*. This parasite has since been shown to belong to the genus *Onchocerca*; other species of which occur in domesticated animals and in the case of the Australian cattle have given rise to problems of considerable economic importance.

In 1898 a very great advance was made by LOOSS when he demonstrated that the hook-worm *Ancylostoma duodenale* entered the body through the skin and migrated via the blood vessels, heart, lungs, trachea, and œsophagus to its habitat in the duodenum.

In 1899 BLANCHARD collected evidence in an attempt to implicate Oxyuris in a catarrhal condition of the appendix and two years later he and KATSURADA gave descriptions of the pathological lesions produced in the bile ducts by the Asiatic liver fluke *Clonorchis sinensis* which had been named in 1875 by COBBOLD.

In 1902 STILES explained a number of discrepancies in the descriptions of earlier workers by showing that there were two species of hook-worms in man, and he clearly differentiated the new *Necator americanus* from *Ancylostoma duodenale*. MANSON reported also a case of Bilharzia from the West Indies in which the infection was entirely intestinal and due to lateral spined eggs.

An important discovery was made in 1904 by KATSURADA who found a new species of Bilharzia worm in cats and man. A few months afterwards CATTO, unaware of KATSURADA'S work, gave a detailed account of the pathological changes in a fatal human case and describes the worms which had been named *Schistosoma cattoi* by BLANCHARD.

BRUMPT of Paris put forward the view that the embryos *Filaria diurna* were the young of the *Filaria loa*. LOOSS experimentally demonstrated upon himself that the larvæ of *Strongyloides stercoralis* of BAVAY (1877) infected by penetrating the skin, thus confirming the work of VAN DURME who had established this point for an allied species in the chimpanzee two years earlier.

In 1904 BENTLEY introduced Betanaphthol as a cure for hook-worm. In 1911 chloroform, which had previously been used for the treatment of tape-worm, was introduced by SCUHLTZ for the cure of hook-worm.

LEIPER in 1912 showed in West Africa that the development of the eye-worm Loa took place in two species of flies of the genus Chrysops.

During a somewhat short period of years a number of relatively rare parasites had been described from man, viz. :—*Physaloptera caucasica* v. LINSTOW, 1902, *Œsophagostomum brumpti* RAILLIET ET HENRY, 1905, *Physaloptera mordens* LEIPER, 1907, *Œsophagostomum stephanostomum* var. *thomasi* RAILLIET ET HENRY, 1909, *Lagochilascaris minor* LEIPER, 1909, *Ancylostoma ceylanicum* LOOSS, 1911, *Metagonimus yokogawai* KATSURADA, 1912, *Syngamus kingi* LEIPER, 1913, and *Trichostrongylus orientalis* JIMBO, 1914.

Far more important than these new accretions to helminthological knowledge were the widespread efforts which were being made throughout the tropics to cope with the problem of hook-worm disease.

In 1909 a new chapter was opened in the history of disease prevention by the appointment of the Rockefeller Sanitary Commission to study the problem of hook-worm control. As a result of its investigations and that of the International Health Board established in 1913 by MR.

ROCKEFELLER with an endowment of one hundred million dollars, hookworm infection as a disease has been eradicated from many countries, although as an infection its disappearance can only follow upon years of sustained co-operation in public sanitation by the inhabitants of infected areas.

The nature of calabar swellings was discussed by MANSON in 1910 and linked with the subcutaneous migrations of the immature adult Loa. In 1911 KOBAYASHI had succeeded in transmitting to goats, dogs and other laboratory animals infections with *Clonorchis sinensis* by feeding them with various cyprinoid fishes in which the cercariæ were encysted. It was not until 1918, however, that MUTO worked out the development of these cercariæ in a mollusc of the genus Bythinia.

Although the Bilharzia worm was one of the first of the flukes to be found in man and was a parasite which subsequent years tended more and more to prove of great pathological importance it was not until the second decade of the twentieth century that any success attended efforts to unravel its life history. Looss in Egypt had from 1895 onwards attempted to implicate various freshwater molluscs in the spread of Bilharzia disease. These attempts proved uniformly negative and he fell back on the hypothesis that infection took place through the skin and that those developmental changes which in other trematodes occurred in the liver of snails were undergone in the human liver by the *Schistosoma hæmatobium*. He assumed also that man was the only mammalian host in which the parasite could attain complete development. The Asiatic Schistosome which had been discovered by KATSURADA in 1904 was soon found to parasitise dogs, cats and cattle. It therefore became a more promising species for experiment than the African form. By immersing cats in water in the infected regions KATSURADA and HASHEGAWA were able to induce heavy infestations through the skin. In 1913 MIYAIRI briefly announced successful infection of an intermediate host with the embryos hatched from the eggs of *Schistosoma japonicum*. In the following year MIYAIRI and SUZUKI described the developmental forms obtained experimentally. LEIPER and ATKINSON infected rats from naturally infected snails and confirmed MIYAIRI's results in general. In 1915 LEIPER studied the problem of Bilharzia infection in Egypt, and showed that the disease there was due to two distinct species of Bilharzia worms which were

conveyed by different species of freshwater mollusc. In 1918 CHRISTOPHERSON introduced antimony tartrate for the treatment of Bilharzia disease and in the same year DIAMANTIS showed that successful results could also be obtained by the use of emetine.

In 1914 also the source of human infestation by the lung fluke *Paragonimus ringeri* was discovered by NAKAGAWA, who implicated certain species of freshwater crabs as second intermediate host harbouring the infective encysted cercaria.

In 1916 unexpected light was thrown on the pathology and life-cycle of *Ascaris lumbricoides* by STEWART, who showed from experiments on rats that after hatching in the gut the larvæ of this parasite migrate into the liver, thence to the lung, causing considerable local disturbances in the course of its migrations through this organ before it returns via the trachea and œsophagus to its final habitat in the intestines.

In 1922 KOINO showed that the same migration and pulmonary symptoms follow the swallowing of the embryonated eggs by man. In 1917 the life-cycle of *Dibothriocephalus latus* was completed for the first time, ROSEN showing by experiment that a cyclops is an essential first intermediary for this tape-worm before the larval stage in fish can be reached. In the same year YAMADA and YOSHIDA fed a *Ligula mansoni* from a human case to a dog and found that it developed into a species of Dibothriocephalus distinct from *Dibothriocephalus latus*.

In 1919 one of those strange errors was made which appear to befall even the most careful of observers. A large number of cases of infection with a new species *Oxyuris incognita* were put on record. These, four years later (1923), were shown by SANDGROUND to be the eggs of *Heterodera radicicola*, an eel-worm which commonly infests a number of food plants. 1919 was notable, however, for the important discovery by FAIRLEY of a specific test for Bilharzia infection. Using the infected liver of snails as antigen for complement deviation testing he was able to ascertain the existence of Bilharzia infection even prior to the appearance of eggs in the excreta.

In the succeeding seven years several further life-histories were elucidated. In 1920 NAKAGAWA showed that two species of *Planorbis* were intermediary hosts for the large intestinal fluke, *Fasciolopsis buski*. In 1923 ONDI and NISHIO demonstrated that the Heterophyes worm was acquired from the fish *Mugil cephalus*. BLACKLOCK in 1926 showed

that *Onchocerca volvulus* was transmitted by a *Simulium damnosum*, while in the following year Dyce Sharp similarly demonstrated that *Filaria perstans* was spread by *Culicoides austeni*.

The most recent and perhaps one of the most remarkable facts concerning the invasion of man by worm parasites has been recorded by Faust (1928). It appears that the *Ligula mansoni* enters man through the application of split frogs to wounds as a native method of treatment, the Ligules migrating from the frog poultices into the human body.

In this historical review I have dealt chiefly with the discoveries of the parasites and of their life histories, a knowledge of which is essential for the prevention of infection. Only occasionally has reference been made to the host reactions and the symptoms induced by these parasites. A consideration of these has been largely omitted on account of the lack of time at my disposal.

I must in conclusion make reference, however, to certain notable advances which have recently been made in the subject of Anthelmintic medication. Largely due to the influence of American workers, the earlier empirical methods of judging of the success of a drug from consideration only of the worms removed is being abandoned in favour of a method of critical testing whereby the number of worms left after treatment was also taken in consideration. These tests, necessarily carried out on lower animals has led recently to the discovery of certain correlations between the efficacy of anthelmintics and their chemical composition. Thus the chemical series ethylene chloride ($C_2H_4Cl_2$), Chloroform ($CHCl_3$), carbon tetrachloride (CCl_4), and tetrachlorethylene (C_2Cl_4), show in a general way increasing anthelmintic efficacy against hook-worms which is correlated with the increasing chlorine content of these compounds. In addition, however, factors of solubility affect the safety and efficacy of these drugs. The new method, however, has fully justified itself in the hands of Hall, to whom we are indebted for the introduction in 1921 of carbon tetrachloride and in 1926 of tetrachlorethylene, two new drugs which had never before been used as anthelmintics. Carbon tetrachloride, which was largely in chemical use as a fire extinguisher, has already been administered since its introduction to over two million cases of human hook-worm disease while its efficacy in the treatment of liver fluke in sheep has been proved since 1926 by Montgomerie, and recently its use in Bilharzia infection has

been advocated by CAWSTON.

On the zoological side the field of medical helminthology has been fairly fully explored. The main lines for the prevention of the chief diseases have been clearly indicated.

There remains, however, for the future the far more difficult fields of helminth physiology and of host immunity in which the problems would appear to present important differences from those with which the bacteriologist is at present familiar.

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Further Observations on the Morphology of *Heterodera schachtii*, with Remarks on the Bionomics of a strain attacking Mangolds in Britain.

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Introduction.

In a previous publication the morphological characters of a strain of *H. schachtii* specialised on the potato in Lincolnshire were discussed. The size and shape of the brown cysts, size of the eggs and first stage larvæ and the general characters of the adult males of this strain were compared with those of a strain attacking potatoes in Rostock, Mecklenburg, for which the specific name *H. rostochiensis* was suggested by Wollenweber 1923.

Considerable dimensional variations were found to exist in the Lincolnshire strain, and a study of the literature on the morphological characters of *H. schachtii* specialised on sugar-beet and oats, showed equally wide divergencies between the findings of different workers. A certain amount of preserved material of infected roots of beet and oats being available to the writer, as many developmental stages as possible were extracted from these, on which similar morphological studies were carried out. Thus a fairly complete comparative survey of *H. schachtii* specialised on beet, oats and potatoes was completed, and the morphological variations between the strains were found to be insufficient to justify the separation of the strain specialised on potato from those on beet and oats. The name *H. rostochiensis* was, therefore, deemed a synonym for *H. schachtii*.

Subsequent to this publication a quantity of cysts of strains of *H. schachtii* parasitic upon beet and oats have been obtained, and, by

means of these, infections have been established upon these host plants, which have provided fresh material for further study. Abundant fresh material has also been obtained of the Lincolnshire potato-strain, the adult males of which, like those of the beet- and oat-strains, were previously described from preserved material only. Another potato-strain occurring in Lancashire has been obtained, and, in addition to these, two other indigenous strains, one parasitic upon hops, the other attacking mangolds, cauliflowers, and a number of common weeds, have also come under observation.

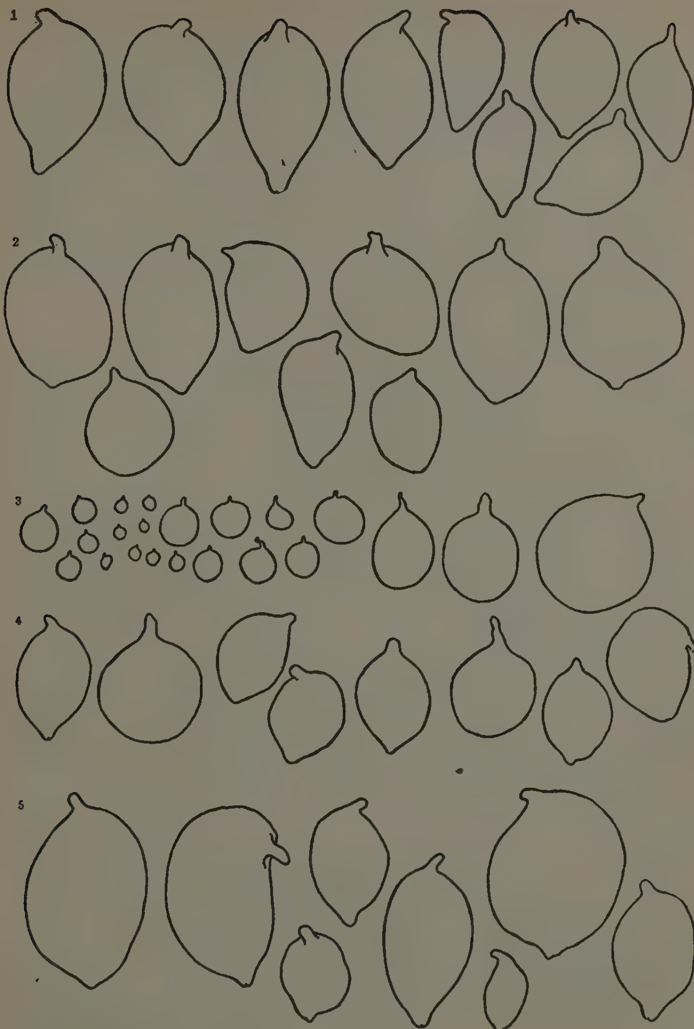
The brown cysts of the beet- and oat-strains, which were not previously available, are described below together with a revision of certain measurements of other stages of these and the potato-strain based on living material. The morphology of the hop- and mangold-strains, with some field observations and cultural experiments on the latter are also described, and some possible causes of morphological variation are discussed.

I should like to express my thanks to Dr. N. A. Kemner and Dr. B. Rademacher who kindly provided me with the oat- and beet-strains already referred to.

Morphology of the Brown Cysts.

Beet strain.—The brown cysts of the beet-strain obtained from Halle-Salle, Germany, were uniformly lemon-shaped, showing the vulva as a distinctly visible projection at the posterior end. They varied from 0·56 by 0·25 mm. to 0·85 by 0·58 mm. in size, the average size being 0·73 by 0·43 mm. They were, therefore, smaller than the cysts of strains described by Voigt, Strubell and Chatin, while falling within the maximum and minimum measurements given by Marcinowski. They differed from Marcinowski's strain, however, by being relatively slightly more elongated and shorter-necked.

Oat strain.—In the Swedish oat-strain, the cysts, while still showing the position of the vulva, were more ovoid in contour than those of the beet-strain. They varied from 0·53 by 0·35 mm. to 0·85 by 0·63 mm. in size, with an average size of 0·71 by 0·50 mm. Thus, while approximately the same in size as those of the strain studied by Voigt, they exceeded Marcinowski's strain in both minimum and maximum dimensions, and were, moreover, relatively broader and shorter-necked than the latter.



1. Cysts of Beet-strain (Halle-Salle).
2. Cysts of Oat-strain (Sweden).
3. Cysts of Potato-strain (Hertfordshire experimentally infected plot).
4. Cysts of Hop-strain.
5. Cysts of Mangold-strain developed on Mangold. (× 33 approx.)

Potato strain.—Further observations on the small cysts occurring in a potato-strain transplanted from Lincolnshire to Hertfordshire soil have been carried out, and these are referred to below. The dimensions of the cysts in Lincolnshire soil were, as previously given, 0·132 by 0·109 mm. to 0·955 by 0·8 mm., and in Hertfordshire soil 0·05 by 0·036 mm. to 0·477 by 0·382 mm. After the 1928 season, however, the maximum size of the cysts present in the infected plot in Hertfordshire was found to have increased to 0·91 by 0·69 mm., possibly the result of a favourable season and improved soil conditions. The length of neck, and ratio of length over length of neck, erroneously given in the previous paper are corrected in the table of dimensions given below.

Hop strain.—In this strain the shape of the cysts was found to vary, the majority being ovoid, with a distinct vulva, resembling in form those of the oat-strain, but a small percentage being rounded, and more closely approximating to the type of cyst produced on the potato.

The variation in size was comparatively slight, but this must have been due in part to the comparatively small number of cysts examined, owing to the scarcity of material. Of fifty cysts which were measured, the largest was only 0·695 long by 0·571 mm. broad, and the smallest 0·333 long by 0·295 mm. broad. The average size of these fifty cysts was 0·534 by 0·402 mm. As in other strains, a wide variation was observed in the length of the neck. While in some individuals the neck was only 0·040 mm. in length, others were found in which the neck measured up to 0·167 mm. These long-necked forms were, however, exceptional, as is shown by the average length of only 0·07 mm.

Mangold strain.—The brown cysts of this strain were uniformly of the lemon-shaped type, with a very distinct posterior projection bearing the vulva.

They showed a wide range in size and exceeded in maximum dimensions the size of cyst formed by any other strain which has been studied by the present writer. The smallest cyst observed measured 0·43 by 0·2 mm., and the largest 1·1 by 0·68 mm., while the average size was found to be 0·84 by 0·51 mm. Thus the range of variation was greater than in the beet-, oat- and hop-strains, but less than in the potato-strain in either Lincolnshire or Hertfordshire soil. The general body-form closely resembled that of the beet-strain as shown by the ratios of the various parts given here in tabular form.

Host.	Length. / Thickness.			Length of Neck in mm.			Length. / Length of Neck.		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Beet (Halle-Salle) ...	1·4	2·2	1·69	0·06	0·12	0·086	6·9	10	8·6
Oats (Sweden)...	1·2	1·7	1·4	0·05	0·11	0·084	7	11	8·8
Potato (Lincs.)	1·06	1·76	1·37	0·014	0·14	0·107	3·7	8·8	5·7
Hop	1·08	1·76	1·36	0·04	0·167	0·07	5·12	10·3	8·47
Mangold ...	1·32	2·16	1·62	0·06	0·14	0·077	5·55	17·0	9·91

Morphology of the Eggs.

Beet strain.—The average size of the eggs extracted from fresh brown cysts was found to be considerably larger than that of the eggs previously obtained from white cysts adhering to preserved roots. The former showed an average size of 0·114 by 0·045 mm., while in the latter the average size had been found to be only 0·088 by 0·04 mm.

That this difference was not entirely the result of shrinkage caused by fixation is, however, indicated by two facts ; firstly, that the smaller size, that of the original strain, corresponds very closely with the measurements given by Strubell and Chatin, both of whom found the beet-strain nematode to possess eggs measuring 0·08 by 0·04 mm. ; and secondly, by a comparison of the maximum and minimum dimensions in the case of the preserved and fresh individuals. In the preserved material the eggs were found to vary from 0·073 by 0·027 mm. up to 0·123 by 0·05 mm., while in the new brown cysts they varied from 0·105 by 0·04 mm. up to 0·125 by 0·05 mm. Thus, although the minimum size of the preserved eggs was much smaller than that of the living eggs, the maximum sizes differed by only 0·002 mm. in length.

This seems to indicate that the differences in average size in these two cases was due, not to shrinkage, but to inherent tendencies in the two strains from which the material was derived, and further emphasizes the suggestion that modifications occurring in this species should not be regarded as being specific in character.

Oat strain.—Similar results were obtained by comparing the eggs of fresh and preserved cysts of the oat-strain, but in this case the living

eggs exceeded equally in maximum, minimum and average dimensions those from the preserved cysts. No previous findings on the size of the eggs of *H. schachtii* specialised upon oats occur in the literature, but, by analogy with the beet-strains, the dimensional divergencies may be assumed to be true modifications.

The eggs of the fresh brown cysts varied from 0·1 by 0·03 mm. to 0·115 by 0·05 mm., with an average size of 0·106 by 0·041 mm. While from the preserved material they varied from 0·073 by 0·025 mm. up to 0·091 by 0·032 mm., with an average size of 0·08 by 0·028 mm. The new strain from oats had, therefore, eggs intermediate in average size between those of the two beet-strains.

Potato strain.—Since the eggs from the Lincolnshire potato-strain, whose size was given in the original publication, were obtained from unfixed cysts newly isolated from soil, no revision of these figures need be made. Measurements of the eggs of the Lancashire strain having been taken, it was found that, as in the cases of the beet- and oat-strains, the eggs of the nematode from the second source exceeded in size those from the first.

The eggs of the Lancashire strain varied from 0·080 by 0·04 mm. up to 0·12 by 0·06 mm., with an average size of 0·11 by 0·055 mm. The differences in size between the eggs of these two strains are less than the differences between the beet-strains and the oat-strains, but it again serves to emphasize the variations which may exist between strains specialised upon a single species of host plant.

Hop strain.—The eggs of the hop-strain varied from 0·075 by 0·035 mm., to 0·1 by 0·055 mm., the average size being 0·089 by 0·042 mm. They thus most closely resembled the original strains of preserved material from oats and beet, and were smaller than the eggs of the potato-strains and the fresh beet- and oat-strains.

Mangold strain.—The eggs of the mangold-strain, on the other hand, were of a larger type, and approximated in size to those of the Halle-Salle beet-strain and Swedish oat-strain. They measured from 0·095 by 0·04 mm. up to 0·125 by 0·05 mm., the average size being 0·112 by 0·046 mm.

Morphology of the First Stage Larvæ.

Beet strain.—The first stage larvæ of the two beet-strains also showed dimensional differences. Those from the preserved material had been

found to possess an average length of 0·29 mm. with the stylet of an average length of 0·025 mm. In the Halle-Salle strain, the length of the larva was found to average 0·457 mm. with the stylet 0·024 mm. Thus the latter, while greater in body-length, had, in the average a shorter stylet. The minimum and maximum body-lengths of 0·24 mm. and 0·318 mm. for the original strain, and 0·43 mm. and 0·50 mm. for the new strain were roughly proportional with one another and with their respective averages.

Comparison with the findings of Strubell and Chatin who gave the length of the larva as 0·36 mm. and 0·35 mm. with stylet lengths of 0·023 mm. and 0·022 mm. respectively, show that, while the present writer's original beet-strain had shorter larvæ with longer stylets, in the Halle-Salle strain the larvæ are longer but have stylets intermediate in length between those of Strubell's and Chatin's strains and the writer's original strain.

It seems probable that the body-length of the preserved larvæ was to some extent affected by the reagents used as fixatives, although, as already demonstrated for the potato strain, the length of the stylet does not necessarily vary proportionally with the length of the larva.

Oat strain.—As in the beet-strain, the first stage larvæ from the new oat-strain were considerably larger in minimum, maximum and average sizes than were the larvæ from the preserved material, and again the average length of the stylet was found to be smaller in the living specimens.

The body-length of these larvæ was found to vary from 0·43 to 0·50 mm., average length 0·47 mm. and average length of stylet 0·022 mm. In the preserved material the corresponding measurements were 0·22 to 0·317 mm., average 0·289 mm., and average length of stylet 0·027 mm. Again a certain amount of shrinkage had probably taken place in the original specimens.

Potato strain.—The larvæ of the Lancashire potato-strain varied in length from 0·35 to 0·52 mm., with an average length of 0·46 mm. That is, the larvæ, like the eggs, were slightly larger in this strain than in the Lincolnshire strain. The average lengths of the stylets of these two strains were, however, identical, both measuring 0·023 mm.

Hop strain.—Like the eggs, the first stage larvæ of this strain were slightly smaller than those of the potato-strains and the new beet- and

oat-strains. They measured 0·316 to 0·48 mm. in length, with an average length of 0·39 mm., and the stylet showed an average length of 0·023 mm. The general morphological characters showed no variation from those of the other strains.

Mangold strain.—The first stage larvæ of the strain parasitic upon mangolds showed a variation in length of from 0·45 to 0·59 mm., the average length being 0·50 mm. and stylet length 0·026 mm. The length of the larva therefore, as in the hop-strain, was proportional with the size of the egg, and in this particular again the mangold-strain exceeded any of the other strains in size.

Morphology of the Adult Male.

Technique.—The males from beet-, oat- and potato-strains whose dimensions were given in the earlier paper, were obtained from preserved roots by means of dissection. That a considerable amount of shrinkage had taken place in these specimens may be concluded by a comparison of these figures for the potato-strain with the new measurements given below. In addition to this, it seems possible that another error may have been introduced by a small percentage of immature males, liberated by rupture of the sheath during dissection, being included amongst those measured, although care was exercised to prevent this occurrence.

To avoid this difficulty with the fresh material, a different method of isolating the males was adopted. Soil from around the roots of the infected plants was removed and placed in a Baermann apparatus. After from twelve to twenty-four hours the nematodes extracted from the soil were drawn off. By this method mature males which had become free in the soil were secured without the admixture of developmental forms which remained attached to the roots.

Beet strain.—The lengths of the males of the nematode strains recorded by Strubell, Chatin and Marcinowski, and the dimensions of the males obtained by dissection from preserved roots by the present writer, have already been published in tabular form and need not be repeated here. The body-length was found to be uniformly less in the writer's fixed specimens than the lengths given by previous observers, while the average lengths of stylet and spicules of the preserved strain were slightly greater.

In the new strain the males were found to measure from 1·32 mm. up to 1·63 mm. in length. The average length was 1·46 mm. and the

average ratio of length over breadth was 37. The average lengths of stylet and spicules were 0·028 mm. and 0·034 mm. respectively. Hence it will be seen that the males of the Halle-Salle strain were notably longer than those of any previous records, the largest of which, a beet-strain (Marcinowski 1909) measured only 1·37 mm. The body-form, as indicated by the ratio of length over breadth, was broader than in Marcinowski's strain, more nearly approaching Strubell's findings to which the length of stylet and spicules also roughly corresponded. It should be noted that the latter were shorter in the new strain than in the preserved material.

Oat strain.—Similar results were obtained with the oat-strain, where again the body-length was greater, and the stylet and spicules were somewhat shorter in the new material than in the old, but in this case the body was proportionally narrower.

The dimensions of the males of the Swedish oat-strain were as follows :—Minimum length, 1·24 mm. ; maximum length, 1·48 mm. ; average length, 1·36 mm ; ratio of length over breadth, 39 ; average length of stylet, 0·028 mm. ; average length of spicules 0·036 mm.

Potato strain.—Males of the Lincolnshire potato-strain isolated from soil in the manner described above, varied in length from 0·91 mm. up to 1·23 mm., with an average length of 1·13 mm. The average ratio of length over breadth was 28, and the stylet and spicules averaged 0·026 mm. and 0·039 mm. in length respectively.

That is, maximum, minimum and average figures all exceeded those compiled from fixed specimens, while the latter were also rather more slender in form than the living individuals. The stylet and spicules were again found to be shorter than in the preserved individuals. In the former cases, this difference is attributable to the fact that completely different strains, from widely separated localities were under observation. In the present case, however, the same strain as was formerly used is under discussion. It seems unlikely that any great shrinkage in hard structures such as stylet and spicules would be brought about by fixation, and the difference can, therefore, only be attributed either to the natural range of variation of the parasite, or to slight changes in environmental conditions such as the variety of the host plant, or, more probably, soil conditions. In this relation it should be noted that the first series of specimens were derived from roots grown in Lincolnshire soil, while

the second series were extracted from Hertfordshire soil. Unfortunately no males of the Lancashire strain were available for comparison.

Hop strain.—The males of the hop-strain had an average length of only 0·85 mm., ranging between minimum and maximum lengths of 0·71 mm. and 0·962 mm. The body-form, as denoted by the ratio of length over breadth resembled that of the fresh specimens of the potato-strain, this ratio for the hop-strain being 29. The stylet and spicules were also rather short, having average lengths of 0·027 mm. and 0·032 mm. respectively.

Since only a single infected plant was available the range variation indicated by these figures is probably less than naturally occurs within the strain.

Mangold strain.—In this strain the males varied in length from 1·22 mm. to 1·59 mm., and had an average length of 1·42 mm. They were, therefore, intermediate in size between those of the Swedish oat-strain and the Halle-Salle beet-strain, being longer in maximum and average lengths than the former, and slightly shorter than the latter. They were, however, comparatively more slender than the males of either of these strains, since the ratio of length over breadth gave the high average figure of 43. The average length of the stylet was 0·029 mm., and that of the spicules 0·034 mm. These figures also closely approximate to those already quoted for the Halle-Salle beet strain.

The Morphology of the Larvæ and Males expressed by Formulæ.

Since the lengths of the larvæ and males of the strains attacking different host plants was found to vary considerably, while no modifications of the internal structure could be detected by ordinary microscopical examination, it was thought advisable to compare the proportions which the various parts of the body bore towards one another, in order to determine whether any such variations which might serve to differentiate the strains did, in fact, exist. Accordingly, series of measurements were taken, and formulæ drawn up after the manner instituted by Cobb, for the first stage larvæ and males of each strain. The lengths from the anterior end to the base of the lips, the posterior end of the stylet, the posterior end of the œsophageal bulb, and the anus, expressed as percentages of the length, are represented by the figures above the line. The breadths of the nematode at these points, also expressed as percentages

of the length are given below the line, and the terminal figure represents the total body-length in millimeters.

It was, of course, impossible to carry out these measurements for as large a number of individuals as was used in determining the original series of measurements, and the body lengths given in the average formulæ do not, for this reason, correspond exactly to the average figures previously quoted. The nematodes from which the formulæ were drawn up were drawn immediately after being killed by the application of gentle heat.

The average formulæ for the first stage larvæ parasitic upon different species of host plant are as follows :—

$$\text{Beet (Halle-Salle)}\quad \frac{0\cdot9 \qquad 5\cdot9 \qquad 18\cdot0 \qquad 89\cdot6}{2\cdot0 \qquad 3\cdot8 \qquad 4\cdot5 \qquad 2\cdot7}\quad 0\cdot45 \text{ mm.}$$

$$\text{Oats (Sweden) ...}\quad \frac{0\cdot82 \qquad 5\cdot35 \qquad 15\cdot55 \qquad 87\cdot7}{1\cdot95 \qquad 3\cdot5 \qquad 3\cdot85 \qquad 3\cdot1}\quad 0\cdot462 \text{ mm.}$$

$$\text{Potato (Lincs.)...}\quad \frac{0\cdot95 \qquad 5\cdot25 \qquad 14\cdot2 \qquad 89\cdot6}{2\cdot0 \qquad 3\cdot35 \qquad 3\cdot45 \qquad 2\cdot6}\quad 0\cdot47 \text{ mm.}$$

$$\text{Hop ... ...}\quad \frac{0\cdot85 \qquad 5\cdot2 \qquad 15\cdot7 \qquad 89\cdot2}{1\cdot9 \qquad 3\cdot4 \qquad 3\cdot9 \qquad 2\cdot5}\quad 0\cdot48 \text{ mm.}$$

$$\text{Mangold... ...}\quad \frac{0\cdot87 \qquad 5\cdot97 \qquad 18\cdot1 \qquad 91\cdot5}{1\cdot9 \qquad 3\cdot5 \qquad 4\cdot4 \qquad 2\cdot5}\quad 0\cdot468 \text{ mm.}$$

In drawing up these average formulæ considerable differences were found to occur between the measurements of individuals belonging to the same strain. As a means of expressing this range of individual variation a second series of formulæ has been compiled, as follows :—

$$\text{Beet ... ...}\quad \frac{0\cdot7\text{–}1\cdot0 \qquad 4\cdot8\text{–}6\cdot6 \qquad 16\cdot1\text{–}20\cdot2 \qquad 89\cdot3\text{–}90\cdot0}{1\cdot9\text{–}2\cdot2 \qquad 3\cdot7\text{–}4\cdot1 \qquad 4\cdot5\text{–}\ 4\cdot6 \qquad 2\cdot5\text{–}\ 2\cdot9}\quad 0\cdot43\text{–}0\cdot48 \text{ mm.}$$

$$\text{Oats ... ...}\quad \frac{0\cdot7\text{–}0\cdot9 \qquad 4\cdot8\text{–}5\cdot6 \qquad 14\cdot4\text{–}16\cdot5 \qquad 85\cdot1\text{–}88\cdot9}{1\cdot8\text{–}2\cdot1 \qquad 3\cdot2\text{–}3\cdot7 \qquad 3\cdot2\text{–}\ 4\cdot5 \qquad 2\cdot7\text{–}\ 3\cdot5}\quad 0\cdot43\text{–}0\cdot48 \text{ mm.}$$

$$\text{Potato ...}\quad \frac{0\cdot9\text{–}1\cdot0 \qquad 5\cdot0\text{–}5\cdot4 \qquad 10\cdot9\text{–}16\cdot1 \qquad 88\cdot7\text{–}90\cdot4}{1\cdot9\text{–}2\cdot2 \qquad 3\cdot1\text{–}3\cdot7 \qquad 2\cdot2\text{–}\ 4\cdot2 \qquad 2\cdot5\text{–}\ 2\cdot8}\quad 0\cdot44\text{–}0\cdot48 \text{ mm.}$$

$$\text{Hop ... ...}\quad \frac{0\cdot8\text{–}0\cdot9 \qquad 4\cdot9\text{–}5\cdot5 \qquad 14\cdot8\text{–}16\cdot4 \qquad 88\cdot8\text{–}89\cdot6}{1\cdot8\text{–}2\cdot0 \qquad 2\cdot9\text{–}3\cdot6 \qquad 3\cdot4\text{–}\ 4\cdot3 \qquad 2\cdot4\text{–}\ 2\cdot7}\quad 0\cdot45\text{–}0\cdot52 \text{ mm.}$$

$$\text{Mangold ...}\quad \frac{0\cdot8\text{–}0\cdot9 \qquad 5\cdot9\text{–}6\cdot0 \qquad 17\cdot5\text{–}19\cdot1 \qquad 90\cdot1\text{–}93\cdot2}{1\cdot7\text{–}2\cdot0 \qquad 3\cdot3\text{–}3\cdot7 \qquad 4\cdot2\text{–}\ 4\cdot6 \qquad 2\cdot0\text{–}\ 3\cdot1}\quad 0\cdot46\text{–}0\cdot48 \text{ mm.}$$

These figures show that the labial region in the larvæ of the potato-strain is rather more strongly developed than in the larvæ of the other strains. In the hop-, oat- and potato-strains the lengths from the anterior

end of the body to the base of the stylet and to the posterior end of the œsophageal bulb is proportionally less than in the beet- and mangold-strains, while the thickness of the body at this latter point also tends to be slighter. The larvæ of the mangold-strain differ from those of the beet-strain, however, in being shorter in the post-anal region. All of these differences are borne out both by the average and the variational formulæ.

Since fixed larvæ only, of the original beet- and oat-strains were available it was thought inadvisable to compare the proportions of these with the fresh material of the German and Swedish strains, but larvæ of the Lancashire potato-strain were measured and found to give the following formulæ :—

$$\frac{1\cdot0 \qquad 5\cdot9 \qquad 19\cdot4 \qquad 87\cdot8}{2\cdot32 \qquad 3\cdot8 \qquad 4\cdot4 \qquad 2\cdot5} \; 0\cdot41 \text{ mm.}$$

$$\frac{1\cdot0\text{–}1\cdot1 \qquad 5\cdot6\text{–}6\cdot3 \qquad 15\cdot0\text{–}22\cdot4 \qquad 86\cdot6\text{–}88\cdot5}{2\cdot2\text{–}2\cdot5 \qquad 3\cdot7\text{–}4\cdot0 \qquad 3\cdot9\text{–}4\cdot9 \qquad 2\cdot3\text{–}2\cdot8} \; 0\cdot35\text{–}0\cdot45 \text{ mm.}$$

This strain, therefore, shows the labial region even more strongly developed than in the Lincolnshire strain, but the distances from the anterior end to the base of the stylet and the posterior end of the œsophageal bulb are longer than in the Lincolnshire strain, and in these particulars the Lancashire strain resembles the beet- and mangold-strains rather than the oat- and hop-strains.

Thus the size of the lips of the potato-strains and the shortness of the post-anal region in the mangold-strain remain the only two distinguishing features.

The average formulæ for the adult males and the range of variation occurring between individuals of these strains were as follows :—

Beet (Halle-Salle)...	$\frac{0\cdot44 \quad 2\cdot12 \quad 6\cdot8 \quad 99\cdot55}{0\cdot73 \quad 1\cdot3 \quad 1\cdot8 \quad 1\cdot11}$	1·457 mm.
Oats (Sweden) ...	$\frac{0\cdot42 \quad 2\cdot15 \quad 8\cdot3 \quad 99\cdot42}{0\cdot85 \quad 1\cdot62 \quad 2\cdot15 \quad 1\cdot07}$	1·36 mm.
Potato (Lincs.) ...	$\frac{0\cdot47 \quad 2\cdot3 \quad 8\cdot35 \quad 96\cdot72}{0\cdot9 \quad 1\cdot9 \quad 2\cdot42 \quad 1\cdot12}$	1·17 mm.
Hop	$\frac{0\cdot57 \quad 3\cdot37 \quad 9\cdot91 \quad 99\cdot35}{1\cdot15 \quad 2\cdot15 \quad 2\cdot67 \quad 1\cdot57}$	0·85 mm.
Mangold	$\frac{0\cdot40 \quad 1\cdot9 \quad 7\cdot7 \quad 99\cdot2}{0\cdot7 \quad 1\cdot35 \quad 1\cdot7 \quad 1\cdot3}$	1·48 mm.
Mangold Strain on Cauliflower ...	$\frac{0\cdot42 \quad 2\cdot4 \quad 9\cdot1 \quad 99\cdot4}{0\cdot82 \quad 1\cdot52 \quad 2\cdot1 \quad 1\cdot35}$	1·24 mm.

Beet	$\frac{0\cdot39–0\cdot51}{0\cdot63–0\cdot8}$	$\frac{1\cdot9–2\cdot2}{1\cdot1–1\cdot4}$	$\frac{6\cdot1–7\cdot6}{1\cdot5–2\cdot2}$	$\frac{99\cdot5–99\cdot6}{1\cdot01–1\cdot2}$	1·37–1·58 mm.
Oats	$\frac{0\cdot4–0\cdot5}{0\cdot8–0\cdot9}$	$\frac{1\cdot9–2\cdot4}{1\cdot5–1\cdot8}$	$\frac{6\cdot9–9\cdot0}{1\cdot9–2\cdot4}$	$\frac{99\cdot2–99\cdot5}{1\cdot0–1\cdot2}$	1·24–1·48 mm.
Potato	$\frac{0\cdot4–0\cdot6}{0\cdot8–1\cdot0}$	$\frac{1\cdot8–2\cdot6}{1\cdot8–2\cdot1}$	$\frac{7\cdot7–9\cdot0}{2\cdot0–2\cdot6}$	$\frac{95\cdot5–97\cdot5}{1\cdot0–1\cdot2}$	1·11–1·24 mm.
Hop	$\frac{0\cdot5–0\cdot7}{1\cdot0–1\cdot4}$	$\frac{3\cdot2–3\cdot8}{2\cdot0–2\cdot4}$	$\frac{9\cdot0–10\cdot8}{2\cdot4–3\cdot0}$	$\frac{99\cdot2–99\cdot5}{1\cdot1–2\cdot0}$	0·71–0·95 mm.
Mangold	$\frac{0\cdot39–0\cdot42}{0\cdot7}$	$\frac{1\cdot4–2\cdot13}{1\cdot3–1\cdot4}$	$\frac{5\cdot9–8\cdot9}{1\cdot6–1\cdot9}$	$\frac{98\cdot9–99\cdot4}{1\cdot3}$	1·34–1·59 mm.
Mangold Strain on Cauliflower ...	$\frac{0\cdot39–0\cdot49}{0\cdot7–1\cdot0}$	$\frac{2\cdot3–2\cdot4}{1\cdot4–1\cdot8}$	$\frac{8\cdot3–9\cdot8}{2\cdot0–2\cdot2}$	$\frac{99\cdot2–99\cdot6}{1\cdot2–1\cdot5}$	1·22–1·29 mm.

An analysis of these figures shows that in the hop-strain the labial buccal and œsophageal regions are proportionally more strongly developed than in the strains on other hosts. The potato-strain differs from the others in having, in the average, a comparatively longer post-anal region, but the natural variation in the length of this region amongst individuals of the potato-strain is greater than the differences that exist amongst the strains on other host plants. Further, the average lengths from the anterior end to the terminations of the buccal region and œsophageal bulb of the mangold-strain when parasitic on cauliflower, are quite markedly different from these lengths when mangold is the host plant.

A consideration of the variations shown by these formulæ leads to the conclusion that, since the variations in the morphology of individual nematodes of a single strain are so great, and since two strains highly specialised upon the same host-plant, as in the two potato strains, show dissimilar modifications in proportional structure, the proportional relationships between parts of the body should not be considered diagnostic in character for the strains in which they occur.

Field Observations on the Mangold Strain.

This strain came under observation in the late autumn of 1928, and as stated above, was found attacking mangolds, cauliflowers, and a variety of weeds and grasses.

The land on which the infection occurred had been cropped with mangolds in alternate years over a very long period, and had, within the present owner's experience yielded crops diminishing from 40 tons per acre to 15 tons per acre in 1928.

The 1928 crop showed a profuse development of lateral rootlets, such as is typical of sugar-beet when heavily infested with *H. schachtii*. White cysts adhering to the roots were still numerous when the observations were carried out late in October, and, in a portion of the field from which the crop had been lifted, and which was being ploughed, white cysts were abundant and plainly discernible on the newly turned ridges. The late occurrence of the white cysts is of some interest in view of the writer's observations on *H. schachtii* attacking potatoes. Both in Hertfordshire and in Lancashire, where field observations on potato-strains had been carried out, cysts had practically disappeared from the roots of the plants by the beginning of October, and all the cysts present in the soil had turned brown. Further, it had been noted that when, in the early autumn, the roots of a potato-plant bearing white cysts were exposed to the air for 24 hours, all the cysts changed during this time from white to brown. In the mangold strain this change took place much more slowly, for, two days after ploughing, the surface of the soil still showed innumerable white cysts in which no apparent change had occurred.

Cyst extractions taken from soil samples over the whole of the affected area showed a fairly heavy and uniform infection throughout.

The cauliflowers showed a somewhat slighter infection than the mangolds. This crop had also been grown frequently over a long period. Cysts were also found on the following weeds, and it seems probable that other species were also attacked which escaped detection owing to the lateness of the season :—

Matricaria sp. (Mayweed), *Sonchus oleraceus* (Sowthistle), *Lamium* sp. (Dead nettle), *Urtica urens* (Small nettle), *Urtica dioica* (Common nettle), *Rumex* sp. (Dock), *Papaver* sp. ? *rhœas* (Poppy), *Solanum nigrum* (Nightshade), *Veronica officinalis* (Common Speedwell), *Agropyrum repens* (Couch grass), *Poa anua* (Meadow grass), *Lolium perenne* (Rye grass).

In no case was a heavy infection found on these weeds, and although the Nettles, Sowthistle and Dock were found infected over the whole area of the field, of the other species only occasional individuals were found bearing cysts.

Cultural Experiments with the Mangold Strain.

In view of the wide range of host plants found naturally infected with this strain, it was decided to test its power of attacking other plant species, more particularly those of some economic importance. A series of pot experiments was set up in which a variety of vegetable, cereal, grass and weed seeds were grown in infected soil.

Of the cereals, wheat, oats and barley were grown in infected soil for a period of four months. After this time the pots were turned out, the roots carefully examined for cysts, and a quantity of roots and soil placed in a Baermann apparatus to extract any larvæ or males that might have been present. In no case were cysts found and the Baermann extractions showed a very few first stage larvæ but from three extractions performed in the case of each pot no males were procured. It was therefore concluded that no infections of these plants had been brought about.

The following ten species of grasses were similarly tested :—

Dactylis glomerata—Rough cocksfoot.
Festuca pratensis—Meadow fescue.
Festuca elatior—Tall fescue.
Poa trivialis—Rough stalked meadow grass.
Poa pratensis—Smooth stalked meadow grass.
Alopecurus pratensis—Meadow foxtail.
Phleum pratense—Meadow catstail or Timothy.
Lolium perenne—Ryegrass.
Avena elatior—Tall oat grass.
Anthoxanthum odoratum—Sweet vernal grass.

No infections were discovered on these grasses after four months and very few first stage larvæ were extracted from the soil.

Of the weeds grown in infected soil, Poppy and Dock, which had already proved to be susceptible, were included to test the reliability of the method. The other species used were Groundsel, Dandelion, Shepherds Purse and Muckweed. Only two of these species, Dock and Shepherds Purse, became infected in these trials, but on these the infection was heavy.

Twenty-seven species of vegetables were similarly tested and gave the following results :—

1. Onion (*Allium cepa*)—Bedfordshire Champion—Negative.
2. Leek (*Allium porrum*)—Sutton's Improved Musselburg—Negative.

3. Cucumber (*Cucumis sativus*)—Telegraph—Negative.
4. Vegetable marrow (*Cucurbita pepo ovifera*)—Sutton's—Negative.
5. Carrot (*Daucus carota*)—Sutton's Champion Horn—Negative.
6. Parsnip (*Pastinaca sativa*)—Sutton's Student—Negative.
7. Parsley (*Carum petroselinum*)—Sutton's Imperial Curled—Negative.
8. Celery (*Apium graveolens*)—Sutton's Solid White—Negative.
9. Broccoli (*Brassica oleracea botrytis asparagoides*)—Sutton's Favourite—Positive.
10. Cabbage (*Brassica oleracea capitata*)—Sutton's Flower of Spring —Positive.
11. Brussels Sprouts (*Brassica oleracea bullata gemmifera*)—Sutton's Fill Basket—Positive.
12. Cauliflower (*Brassica oleracea botrytis cauliflora*)—Sutton's Autumn Mammoth—Positive.
13. Kale (*Brassica oleracea acephala*)—Sutton's Hardy Sprouting—Positive.
14. Savoy Cabbage (*Brassica oleracea bullata*)—Sutton's Perfection —Positive.
15. Turnip (*Brassica rapa*)—Sutton's Greentop White—Positive.
16. Radish (*Raphanus sativus*)—Mixed Olive—Positive.
17. Lettuce (*Lactuca sativa*)—Sutton's Black-seeded Bath—Negative.
18. Potato (*Solanum tuberosum*)—Sharpe's Express—Negative.
19. Tomato (*Lycopersicum esculentum*)—Klondine Red—Negative.
20. Beet (*Beta vulgaris*)—Sutton's Greentop—Positive.
21. Sugar-beet (*Beta vulgaris*)—Sutton's Improved Sugar—Positive.
22. Mangold (*Beta vulgaris*)—Sutton's Prize Winner Globe—Positive.
23. Spinach (*Spinacia olearacea*)—Positive.
24. Broad-bean (*Faba vulgaris*)—Sutton's Prolific Longpod—Negative.
25. Runner-bean (*Phaseolus multiflorus*)—Sutton's Scarlet—Negative.
26. Dwarf-bean (*Phaseolus vulgaris*)—Sutton's Satisfaction—Negative.
27. Pea (*Pisum sativum*)—Improved Alderman—Negative.

From these results it will be seen that species of the *Liliaceæ*, *Umbelliferæ*, *Compositæ*, *Solanaceæ*, *Cucurbitaceæ and Papilionaceæ* proved uniformly immune from attack, while the *Cruciferæ* and *Chenopodiaceæ* were in every case susceptible.

Although the degree of root development differed so widely as to render the cyst-estimation method useless as a means of comparing the intensity of attack upon the various susceptible species, some idea

of this was gained by the marked difference which existed in the number of males and first stage larvæ extracted by the Baermann method. The soil used for these extractions was approximately equal in volume and was allowed to remain in the Baermann apparatus for the same length of time in every case.

Of the two susceptible families, members of the *Chenopodiaceæ* were the more extensively parasitised. With the exception of spinach, cysts were plentiful on the roots of these plants, and from 25 per cent. to 50 per cent. more males were obtained from the Baermann extractions than from any of the *Cruciferæ*. This result confirms the field observation that Mangolds were more heavily infested than Cauliflowers grown on the same land.

Among the non-susceptible varieties, first stage larvæ were present in numbers in the soil in which the two species of *Liliaceæ*, Onion and Leek, had been grown. They were markedly scarce in the pots containing the *Umbelliferæ*. Free larvæ were present, but not in great number in the soil surrounding the roots of members of the *Solanaceæ* and *Compositæ*, and to a less extent in the soil in which the *Papilionaceæ* and *Cucurbitaceæ* were grown.

Although a single trial is insufficient evidence on which to base the assumption that any species of host is non-susceptible, and it is possible that, as with the oat-strain, some varieties of a single species of host-plant may display a stronger immunity to attack than others, these results, taken in conjunction with the field observations, appear to be of some significance.

Since larvæ were stimulated to hatch in greater numbers by proximity to the growing roots of the *Liliaceæ* than by those of the *Umbelliferæ*, it seems possible that members of the former family might, under favourable circumstances, be attacked. That certain monocots—the grasses, have been found to be parasitised under natural conditions, supports this suggestion. On the other hand, no members of the *Umbelliferæ*, have been found to be affected by the nematode, while a few isolated specimens of the *Solanaceæ* and *Compositæ* were found to bear cysts

That the grasses, cereals and most of the weeds gave negative results in these trials is not taken as conclusive evidence of their immunity, since this could only be established by repeated trials under natural conditions.

Variations in the Morphology of the Males from different Host Plants.

In order to determine whether any morphological changes were induced in the nematode by adaptation to host-plants less favourable to the parasite than the mangold, measurements were made of the adult males found throughout the complete range of experimental host-plants. Not less than ten males were measured from each host-plant, and the results of this survey are shown in the following table :—

Host.	Total length of Male.			Length of Stylet.			Length of Spicules.		
	Min.	Max.	Av.	Min.	Max.	Av.	Min.	Max.	Av.
Sugar Beet ...	1·53	1·72	1·64	0·027	0·030	0·028	0·034	0·038	0·036
Beet	1·37	1·66	1·53	0·028	0·033	0·029	0·030	0·038	0·035
Mangold ...	1·22	1·59	1·42	0·027	0·030	0·029	0·032	0·036	0·033
Spinach ...	1·17	1·49	1·35	0·027	0·032	0·029	0·032	0·036	0·034
Turnip... ...	1·19	1·47	1·39	0·028	0·035	0·033	0·030	0·045	0·035
Savoy Cabbage	1·12	1·48	1·34	0·027	0·031	0·029	0·030	0·035	0·033
Broccoli ...	0·83	1·53	1·33	0·026	0·029	0·027	0·031	0·035	0·034
Kale	1·18	1·41	1·33	0·025	0·029	0·027	0·032	0·035	0·034
Cauliflower ...	1·22	1·29	1·24	0·026	0·028	0·027	0·030	0·036	0·033
Brussels Sprout	1·02	1·39	1·22	0·026	0·029	0·027	0·028	0·034	0·031
Cabbage ...	0·97	1·25	1·16	0·023	0·030	0·026	0·026	0·036	0·031
Radish... ...	0·79	1·31	1·08	0·025	0·029	0·027	0·024	0·036	0·032
Shepherds Purse...	1·09	1·5	1·33	0·025	0·029	0·028	0·033	0·036	0·034
Dock	1·05	1·23	1·16	0·025	0·030	0·027	0·033	0·035	0·034

From this table it will be seen that the average lengths of the males from the *Chenopodiaceæ* were greater than the average lengths of the nematodes taken from cruciferous hosts with the exception of the turnip, the males from which were longer than those from spinach. Further, the nematodes from the *Chenopodiaceæ* are much more constant in length, varying only from 1·17 to 1·72 mm., than are those from the *Cruciferæ*, which show a variation of from 0·79 up to 1·53 mm., a range almost equal to the length of the smallest individual observed

As compared with body-length the stylet and spicules show in both cases a proportional as well as an actual smaller range in size, but again a greater variation is present in individuals taken from Cruciferous hosts.

These facts indicate that some morphological change takes place as a direct response to the favourability of the host plant, and also point to the supposition that the soft portions of the body are more easily affected by the change of environment than are the stylet and spicules, although these also display a slight modification. That the relative proportions of the lengths of various parts of the body differ in individuals developed on diverse species of host plants has already been shown by the formulæ of the males from mangold and cauliflower.

The suitability of the host plant to the parasite may therefore be concluded to exert some influence over the morphological variations of the latter.

Other Factors influencing Morphology.

In order that experimental work on the control of *H. schachtii* parasitic upon potatoes might be carried out, a plot of land in Hertfordshire was infected with the eelworm in 1926. This was accomplished by scattering heavily infected Lincolnshire soil in drills over the desired area, in which potatoes were then planted. Since 1926 potatoes have been cropped regularly on this land and the infection has spread.

In the autumn of 1927 cysts were extracted from a quantity of this soil taken from a portion of the field to which the infection had naturally spread, and measurements of these were compared with those of cysts from Lincolnshire soil. It was found that a marked decrease had occurred in the size of the cysts in the experimental plot. Further, it was found that a large proportion of the cysts from this soil were so small as to be indistinguishable without the aid of a lens, while examinations of large quantities of Lincolnshire soil failed to reveal any of these minute forms. Unfortunately the proportions in which the normal macroscopic and small forms occurred was not at this time determined.

During the 1928 season the potato crop grown on the infected plot showed, for the first time, some evidence of the eelworm attack, and the roots of the plants were seen to be very heavily infested. By manuring and cultivation, the tilth of the soil had by this time been greatly improved.

An examination of the cysts early in 1929 showed that, although the small forms were still present in great numbers, representing over 50 per cent. of the total cyst content, the maximum size of the normal macroscopic cysts had considerably increased, namely from 0·477 by 0·382 mm. in 1928, to 0·91 by 0·69 mm. in 1929. Thus, during the 1928 season, the maximum size of the brown cysts present in this plot had undergone an increase in size of 0·433 mm. in length by 0·308 mm. in breadth, and closely approached the dimensions of the cysts in Lincolnshire soil from which the Hertfordshire strain had originated.

Many of the microscopic cysts were transparent and could be seen to be void of eggs. On dissection, these were found to contain granular, undifferentiated contents. To determine the minimum size of cyst in which eggs were present, and the number of eggs contained by cysts of this strain, a number were dissected and egg counts made.

The smallest cyst in which eggs were found measured 0·31 by 0·17 mm. Many cysts of about this size, and some larger ones were found to be devoid of eggs, containing a granular mass only. The egg counts of the cysts ranging from this smallest size to the largest cyst found are given below.

	Size of Cyst.	No. of Eggs.
1.	0·31 × 0·17 mm.	17
2.	0·34 × 0·25 mm.	11
3.	0·375 × 0·25 mm.	38
4.	0·41 × 0·25 mm.	52
5.	0·48 × 0·3 mm.	52
6.	0·54 × 0·36 mm.	76
7.	0·58 × 0·37 mm.	112
8.	0·65 × 0·58 mm.	424
9.	0·8 × 0·65 mm.	361
10.	0·91 × 0·69 mm.	375

Cysts, light in colour, unwrinkled, and apparently newly formed, were selected for this examination, and although in some cases a few empty egg membranes were present, indicating that some larvæ had escaped, these were included in the counts which are considered to give a fairly accurate representation of the relationship between the size of the cyst and the number of eggs contained.

Discussion.

That considerable ranges in size normally occur between individuals of a single strain specialised upon any host-plant has already been pointed out.

That two strains specialised upon the same species of host in different localities may show even greater variations has been demonstrated by the comparisons between the potato-strains from Lincolnshire and Lancashire, and also between the two oat- and beet-strains whose morphological characters are described above. Soil conditions contribute to some extent in these differences, as has been proved by transferring a Lincolnshire strain to Hertfordshire soil, and this may indeed prove to be the sole factor influencing variations where only one species of host is involved.

In less specialised strains, which attack a comparatively wide range of hosts, modifications in structure may become apparent in relation to the suitability of the host or degree of adaptation achieved by the parasite. This is shown by the modifications of the males from different host-plants which occur in the mangold strain. In the case of the males it has also been shown that such influences are more readily exerted over the soft parts of the body—causing variations in body-length and the proportions of the various organs—than over the stylet and spicules which show only slight variations through a wide range of hosts. Further, when a large number of generations have been produced upon one species of host plant, the range of variation between individuals is less than occurs in the same strain newly transferred to another though closely related species.

From the cultural experiments with the mangold strain it also appears that some natural orders of plants are more subject to attack by a specified strain than are others, although to what extent this indicates the natural tendency of the parasite and how it may be influenced by adaptation to available species does not appear.

The range of host species to which the nematode becomes readily adapted, although varying widely for different strains, appears to be in every case somewhat circumscribed. Where infections are known to exist, a thorough and extensive trial of possible hosts should reveal the varieties of probable hosts, and by this means a system of rotation comprising crops of economic value suitable to the soil and climatic conditions

of the locality, but unlikely to afford the eelworm facilities for spreading should be made possible.

The fundamental relationship of all strains of *H. schachtii* is, however, indicated by the fact that the highly specialised Lincolnshire potato-strain, although unable to attack beet after repeated crops have been grown in the same soil for almost three years, is occasionally found to form a very light infection on the common weed *Chenopodium album*, as reported by Morgan in 1925. Similarly, the mangold strain, which has given a negative result in two attempts to infect potatoes experimentally, was found naturally infecting one individual plant of *Solanum nigrum* out of a large number of plants examined. It seems possible that by transference through such intermediate hosts occurring as weeds on infected land, the range of susceptible host-plants of economic value might be greatly increased, and more especially so when other natural hosts are not available.

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A Note on the identity of the nematode genera *Anguillulina* and *Tylenchus*

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Anguillulina has been revived by Baylis and Daubney (1926) as the correct generic name for those worms which for many years have been considered as belonging to the genus Tylenchus, at least by most specialists on nematodes and plant-pathologists. Gervais and van Beneden on pp. 101 and 102 of the second volume of their work *Zoologie Médicale*, published in 1859, gave a brief account of the eelworm parasite of wheat causing " purples " or " cockles " and that found by Kühn in 1858 in the heads of the fuller's thistle, teasle, both of which they designate as Anguillulines (*Anguillulina*) as follows :—*Anguillulina tritici* and *Anguillulina dipsaci*.

It may be pointed out, in passing, that they were not consistent in the use of the word, for at the end of the description of *A. dipsaci* they spell it as *Anguillula* not *Anguillulina*. This may, however, perhaps be regarded as an unintentional slip or a printer's error. Bastian's paper in which first the name *Tylelenchus* and then *Tylenchus* is applied to members of this genus appeared in 1865 and he designated *Tylenchus davainii* as type species of the genus in a letter to Stiles dated March 22nd, 1904, *vide* Stiles and Hassall (1905).

According to the Law of Priority of the International Rules of Zoological Nomenclature, the earlier name *Anguillulina* must have precedence over the later name *Tylenchus* as the name of the genus. It may be suggested that Anguillulina is very similar to and liable to be confused with Anguillula. Peters (1927), however, has relegated the name Anguillula to the limbo of those forms with an indeterminate type and has adduced very sound reasons for replacing it by the new name *Turbatrix* as the generic name for the vinegar eel, the sour-paste eel and one or two more nematodes. As a result, the possibility of confusion is considerably lessened since the name Anguillula should no longer be used.

The type species of the genus Anguillulina is *A. tritici* (Steinbuch, 1799) and the correct name for *Tylenchus dipsaci* is *Anguillulina dipsaci* (Kühn, 1858).

The writer has recently received a letter from Dr. G. Steiner of the Bureau of Plant Industry, U.S. Department of Agriculture, Washington, on the subject of the names *Anguillulina* and *Tylenchus* in which, whilst admitting that *Anguillulina* is entitled to replace *Tylenchus* on the grounds of priority, the view is taken that because *Tylenchus* is widely used in some of the more popular scientific literature—nursery reports, etc.—and also because there is to-day an extensive literature in which the name occurs, it should be retained.

Accordingly a petition is to be presented to the International Committee on Zoological Nomenclature for the retention of *Tylenchus* as the name of the genus.

In view of the situation which thus arises it is necessary to point out that, for the time being, the question as to which of the two names is to hold the field must be considered as still undecided.

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The Occurrence of *Tylenchus dipsaci* Kühn, in Wild Host Plants in South-West England.

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Introduction.

The plant parasitic nematode, *Tylenchus dipsaci* Kühn, commonly known as the stem eelworm, or alternatively when occurring in narcissus, the bulb eelworm, is a major pest of a wide range of cultivated plants. Accurate knowledge concerning the detailed life-history of the nematode is still of limited extent, despite the large numbers of workers who, throughout Europe and more recently North America, have devoted much time to a study of the subject. In particular it is clear that much remains to be learned concerning the "biologic strain" theory. Investigators, probably without exception, agree that a large number of, so-called, biologic strains of the worm occur. Each of these strains, while morphologically identical with the others, appears to be restricted more or less rigidly to a particular species of host plant.

It is not proposed to consider here the factors governing the formation and maintenance of such strains. Sufficient to say that experimental work, carried out over a number of years by the writer, indicates that at least some of the strains are not entirely restricted to one species of host plant. By way of illustration it may be recalled that an account (3) has been given of a strain which thrived equally well in both oat and in cocksfoot grass, *Dactylis glomerata* L. Recent experiments

indicate that certain strains found originally in weeds are able to transfer to, and more important, to reproduce in, cultivated plants entirely dissimilar from the host plants in which they originally occurred. These experiments need repetition and amplification before any pronouncement can be made concerning the likelihood of extensive transference taking place under natural conditions. Nevertheless, it will be shown that the nematode is commonly present in certain weeds in South-West England, and very probably this would be found to be the case in other areas.

A considerable degree of confidence in the rigidity of " biologic strains " must be felt before exposing cultivated plants, known to be liable to attack, to the risk of regular infection from such sources as the above. This is perhaps particularly applicable to the case of a comparatively valuable crop such as the narcissus, of which a large acreage is grown in the south-west.

The wild host plants of the nematode which are to be considered here are various species of *Plantago* and also *Hypochæris radicata* L. In passing it is desirable to record that cocksfoot grass, *Dactylis glomerata* L., has been found to be attacked in South Devon by the writer, while quite recently Gibson has recorded attacks upon *Allium triquetrum* L. in the Isles of Scilly.

The Seaside Plantain. *Plantago maritima* L.

Previous Records.

The first and apparently only previous record of nematode galls on this plant in Britain is that of Trail (6), who in 1885 described the occurrence in Scotland. Subsequent records are found from various European countries.

Distribution in the South-West.

Galled plants were first observed in Cornwall in April, 1927. A few of these were submitted to Goodey who kindly confirmed the presence of *T. dipsaci* in the galls. The plants were growing at the edge of a sand belt, only a few feet above the tide mark at Marazion in Cornwall. The belt of infested plants extended along the shore for about one mile.

The foreshore is very effectively isolated by the presence of a railway line which runs immediately behind it for the entire length. No galls could be found on other species of plantain which were abundant on the landward side of the rail.

At the time of writing, *P. maritima* has not been found to be attacked by the nematode in any other locality on the Cornish or Devon coast, but opportunity for a thorough survey has not yet occurred.

Symptoms of Attack.

Typical pale-green galls, rounded or oblong in shape, occur at intervals on the leaves and petioles of plants which are comparatively lightly attacked. The individual galls vary in diameter from one to twelve millimetres. When the nematode infestation is heavier the leaves and petioles may be wholly distorted and discoloured. The mid-ribs are frequently distorted, twisted and enormously swollen throughout almost their entire lengths. The whole plant may exhibit marked chlorosis or sometimes a distinct purple to pink discolouration. Numbers of plants have been observed to be killed eventually by the severity of the infestation.

During the summer months the flower stems are also frequently galled and distorted, even to the base of the inflorescence. In spite of this no very marked tendency to blindness, excepting in exceedingly heavily infested plants, has been observed.

Seed Dissemination of the Nematode.

The phenomenon of the seed dissemination of *T. dipsaci* has previously been found in oat by the writer (3) and more recently by Robertson (5). In America Godfrey (2) has recorded it from *Hypochæris radicata*.

In order to determine if this was true also of *P. maritima*, ripening seed from heavily infested plants was examined microscopically. In numerous instances nematodes, in all stages of development including eggs, could be found congregated actually beneath the seed coat. A quantity of ripe seed taken from plants similar to those examined was sown in soil previously sterilised by baking. The percentage germination obtained was singularly low for plantain and the nematodes had clearly injured the embryo in some of the seeds. Nevertheless, over one half of the seedlings successfully raised showed typical injury when only a few weeks old. Examination proved them to be heavily infested

with the nematodes. In numerous cases the first pair of leaves was badly distorted and numbers of the plants succumbed before reaching maturity. Seed from healthy plantains, subjected to the same conditions, showed a considerably higher percentage of germination and the plants produced arrived at maturity almost without exception. The experiment has been repeated on numerous occasions and it is clear that the nematode is regularly distributed in this manner. At the same time, seed from quite heavily infested plants is not found to contain nematodes with the same regularity as in the case of *H. radicata*, which will be discussed later.

The Greater Plantain. *Plantago major* L.

No previous record of the occurrence of *T. dipsaci* in this species appears to exist. Galled plants have occasionally been found at Marazion, Bereferrers and Dawlish Warren, and there is no reason to suppose that the species is materially less prone to attack than are the two considered previously.

The Ribwort Plantain. *Plantago lanceolata* L.

Previous Records.

Galls formed by *Tylenchus* sp. were first recorded by Liebel (4) in 1886. Subsequently several French workers have observed similar injury, but this has apparently escaped notice in Britain until the present time.

Distribution in the South-West.

One or two plants of this species infested with *T. dipsaci* were found, in company with the predominant *P. maritima*, on the shore at Marazion in 1927, and constitute the first definite record of the occurrence in this particular species of plaintain.

Subsequently a locality was found near Bereferrers, South Devon, in which the plantain grew in great profusion. Nearly every plant

Plantago lanceolata L.

Healthy plant on left, contrasted with two infested by *Tylenchus dipsaci*.

throughout an extensive marsh, on the banks of the river Tavy, was more or less heavily infested with the nematodes. The marsh is eight miles from the sea, but is situated on a tidal reach and during the winter months is periodically flooded by brackish water to a depth of some feet. In July, 1928, a small batch of infested plants was found growing in sea-sand only a few feet above the tide mark on Dawlish Warren, South Devon. During the same month further plants, similarly infested, were found in a smallholding devoted principally to the commercial cultivation of narcissi, at Bantham, South Devon. This latter locality is of interest in that it is situated at least one mile from the coast line, is about one hundred feet above sea level and is remote from a stream or river. In September, 1928, similar plants were found commonly on St. Marys, Isles of Scilly, in and around commercial narcissus gardens and in hedges and banks. In some of the cases the isolated positions in which the plants grew clearly suggested a seed-borne origin of the infestations.

From the above it may be seen that there is every indication that the nematode is widely distributed in this host plant, at least in coastal districts, in South-West England. Further it is not without significance that, both in South Devon and the Isles of Scilly, infested plantains have been found on ground devoted to the commercial cultivation of the narcissus, a plant particularly prone to damage by this nematode.

Symptoms of Attack.

The symptoms of attack are, broadly speaking, similar to those exhibited by *P. maritima*. The size of the galls produced is perhaps on the average slightly larger and the crippling of the plants is even more pronounced, at least during the earlier months of the year. By July, plants marked in the spring as being heavily attacked were completely destroyed. On the other hand, numerous plants previously only lightly attacked had made good growth, which almost completely masked the symptoms observed earlier. Generally but little growth took place and a very marked tendency to blindness was apparent, weak sterile flower heads being frequently seen. The tendency to blindness is distinctly less marked in *P. maritima*, in which species quite heavily infested plants usually manage to produce small but fertile seed heads.

Seed Dissemination of the Nematode.

Reference has already been made to the occurrence of isolated infested plants in unlikely places. This can most easily be explained by assuming that the infestation originated in the seed.

As in the previous case, quantities of seed collected from infested plants were sown in baked soil. Numerous nematode infested seedlings were raised in this manner.

The False Dandelion or Cat's-Ear. *Hypochœris radicata* L.

Previous Records.

Trail (6), 1885, again appears to be the first definitely to have recorded nematode galls in this plant, in Scotland. It is, however, possible that Briggs (1) had observed the same phenomenon in South-West England some five years previously, for he writes, "I have once or twice seen a peculiar malformation in this species—concurrent aborted condition of the florets." This is a common symptom of severe attacks. Various continental workers have subsequently recorded the finding of nematode galls on the plant and recently Godfrey (2) has published an account of the finding of *T. dipsaci*, in *Hypochœris radicata*, in North America.

Distribution in the South-West.

Infested plants were first found in small numbers at Marazion, Cornwall, in March, 1928. Six plants only were found, all heavily infested, growing in close proximity to a railway siding. The infestation occurred only one hundred yards from the site on which the nematode was first found in *P. maritima*, but separated from this and the sea by the railway. Extensive search on several occasions revealed no attacked *H. radicata* on the seaward, or *P. maritima* on the landward side of the rails. The identity of the nematode was again kindly confirmed by Goodey.

In May, 1928, a belt of very sandy soil was found on Dawlish Warren, South Devon, in which infested plants grew in profusion. This belt was approximately one thousand yards long by forty yards wide and was situated on the inner side of the Warren, not more than two yards above the tide mark. Plantains grew freely amongst the infested

Hypochæris radicata L.

Flower stems: Nematode infested on left; healthy on right.

plants, but were invariably found to be free from attack. Nevertheless, numerous heavily infested specimens of *P. lanceolata* occurred only two hundred yards from the extreme western end of the belt.

Infested plants have also been found at Lanedon, Cornwall, growing in a bank on arable land several miles from the sea. Oats were growing in the adjacent fields and were not visibly damaged by *T. dipsaci*. At Denham Bridge, South Devon, in a hedgerow near the banks of the river Tavy, attacked plants were commonly found. On St. Marys, Isles of Scilly, infested plants have been found on a commercial narcissus farm and on St. Agnes, isolated infested plants were frequent alongside footpaths. It is therefore clear that, as in the case of *P. lanceolata*, the distribution of plants infested by the nematode is wide.

Symptoms of Attack.

During the greater part of the year, *H. radicata* adopts a flat "rosette" habit of growth. Throughout this period the detection of infested plants is easy. Numerous pale-green galls, very similar in general appearance to those found on plantain, are discernible. Galls are most frequent on the mid-ribs and in these situations are usually elongate. When on the actual blades of the leaves they tend to be more rounded. Plants suffering from unusually heavy infestations frequently exhibit considerable distortion of the leaves, due to the presence of excessively large galls on the mid-ribs, which are thereby curled.

In the late spring and early summer rapid growth of new foliage masks to a large extent the existence of the galls, excepting in plants growing in particularly unfavourable and exposed situations. Long branching flower-stems are produced and, in June and early July, marked distortion and thickening of these, particularly at the points of branching, are to be seen on infested plants. The nematodes congregate and feed in numbers in the bases of the inflorescences and later in the growing seed heads. Practically no tendency to blindness as a result of attack, was ever observed, nor have plants been seen to be killed outright, even when heavily infested and growing in eminently unsuitable locations.

It may be stated that, while the nematode population of individual plants is frequently as large as that found in the plantain, the effect

on the host now under consideration is infinitely less pronounced. This would appear to indicate a high degree of resistence to attack, which might be inherent to the plants, or alternatively have been arrived at as a result of subjection to infestation over a very long period of time.

Seed Dissemination of the Nematode.

That the seed dissemination of the nematode by *H. radicata* is a common phenomenon in North America has already been shown by Godfrey (2). Observations and experiments, by the writer, amply confirm that the same holds good in this country.

Single ripe seeds, taken from infested plants, have on soaking in water, frequently yielded several hundred nematodes of this species. The majority of these were invariably in the immediately pre-adult stage of development, but adults and even eggs also sometimes occur in numbers.

As with plantains, seed from infested plants, when sown in baked soil, produced nematode infested seedlings. The percentage germination of the seed was invariably poor, both from infested and from healthy plants used for control purposes. It is probable that seeds of this plant need a considerably longer resting stage, prior to germinating, than that given. No such difficulty in obtaining germination was ever experienced with plantain seed.

Very considerable significance attaches to the seed dissemination of the nematode by *H. radicata.* The seeds are wind-borne and may, under favourable circumstances, be carried for enormous distances. At the conclusion of the journey fresh foci of infestation may be established. No such wide range of travel is likely, under normal circumstances, in the case of infested plantain seeds.

Conclusions.

It has been established that *Tylenchus dipsaci* is commonly present in various species of *Plantago* and in *Hypochæris radicata*, in South-West England. Infested plantains have invariably been found in close proximity to the coast, or in one case on a tidal reach. It is possible that infestations occur inland also, but extensive search has not revealed this so far, and it may be accepted that coastal infestations are the general rule. Infestations in *H. radicata* are also most numerous

Hypochæris radicata L.

Plant in the winter " rosette " stage. Typical nematode galls may be seen on the mid-ribs of the leaves.

near the coast, but infested plants have sometimes been found inland and remote from running water. It is perhaps permissible to surmise that, while the main foci occur in coastal districts, others have been set up inland by infested wind-borne seeds.

Seed dissemination of the nematode occurs in all the host plants here considered. In *Plantago* spp, and particularly *P. lanceolata*, a tendency towards blindness and in extreme cases death, was observed in infested plants. This suggests that a balance has not yet been arrived at between host and parasite. Further, seeds were by no means invariably found to contain nematodes, even from plants supporting a very large population. In *H. radicata* very large numbers of nematodes were able to thrive without very greatly inpairing the vigour of the plant and a varying degree of seed infestation was the general rule.

In two localities, infested plantains have been found growing actually in ground devoted to the commercial cultivation of the narcissus, a common host of the nematode. In a third locality, infestation occurred on a beach from which seaweed and sand are regularly carted, for the purpose of manuring narcissus beds. A further instance is even more significant. Narcissus plants, *P. lanceolata* and *H. radicata*, all infested by the nematode, were found closely intermingled and it is rather difficult to believe that in this case three separate and distinct " biologic strains " of *T. dipsaci* were at work ; each incapable of attacking either of the other two host plants. As opposed to this, it must be recorded that *Plantago* spp., entirely free from nematodes, were frequently present amongst heavily infested *H. radicata* and *vice-versa*.

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On the Morphology and Biology of a Larval Stage of *Muellerius capillaris* (Mueller, 1889) Cameron, 1927; a Lungworm of Sheep and Goats.

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Introduction.

Muellerius capillaris was first described by Mueller in 1889 who placed it in the genus Pseudalius; it was transferred in 1907 by Railliet and Henry to their new genus Synthetocaulus. In 1927, Cameron, in his review of the family Protostrongylidæ Leiper, 1926, created the genus Muellerius to include this species; a description of this parasite together with figures of the adult and the larval stage commonly met with in the lungs and droppings of sheep and goats, is included in his paper.

M. capillaris inhabits the bronchioles and alveoli of the lungs of sheep and goats but is probably more often observed in the connective tissue of the lungs where it forms nodules of varying sizes. In heavy infestations these nodules are so close together that they appear as large greyish patches on the surface of the lung. The nodules eventually become calcified.

It is not clear to what extent this parasite is pathogenic although it is recorded as a frequent cause of bronchitis and broncho-pneumonia. It would seem, on the other hand, that a sheep can carry a fairly heavy infestation without apparent inconvenience, and one commonly finds

sheep in our abbatoirs which are in good slaughter condition and yet have very patchy lungs.

M. Capillaris is undoubtedly the most common parasite of the lungs of sheep and goats in this country and although these hosts seem to be capable of an appreciable resistance to the parasite, the latter may exert a considerable influence on growth and development.

In the droppings of infected animals only the larvæ of this worm are found since the eggs hatch out in the lung. This larval stage does not develop further in cultures of sheep's fæces and if it is assumed that the parasite has a direct development this can be considered as an infective larva. It differs somewhat, however, from the third stage larva of some intestinal nematodes.

So far, the life history of only one of this group of lung-worms has been worked out, viz., *Ælurostrongylus abstrusus* by Cameron (1927). In this parasite, which is found in the lung of the cat, the larva passes into the mouse to complete its development to the infective stage. The larval stage which passes from the cat is very similar to that found in the lungs of hosts parasitised with *M. capillaris*, but it is unlikely that a rodent can act as an intermediate host in this instance also. Further, there is, so far, no clear evidence against the possibility of *M. capillaris* having a direct development, and experiments to test this possibility are needed before an intermediate host is looked for.

In the following notes some further points in the morphology of the larval stage referred to above are recorded together with the results of experiments on the biology of the larva.

Morphology.

The larva is very small, having a length of 0·30 mm. Cameron (1927) found the larva to be somewhat smaller viz., 0·25 mm. This difference is rather appreciable in so small a worm and it was thought that larvæ teased out from the lung might be smaller than those obtained from droppings. An examination of material from the lung of a sheep gave an average measurement of 0·29 mm., a difference which is too small to suggest the possibility of growth during the passage of the larva through the body. The œsophagus measures 0·16 mm. in length and has two bulbs, the anterior one dividing the œsophagus into the proportion of 8 : 7. The width of the two bulbs varies slightly, the anterior

being 0·007 mm. and the posterior 0·009 mm. The nerve ring is seen immediately behind the anterior bulb and the excretory pore is situated about the same level. Very little could be seen of this latter organ in the majority of the worms other than the small duct leading to the exterior. In one instance, however, this duct could be clearly observed to be passing into a small, oval, excretory cell with a large nucleus. (Fig. 1.)

The width of the body is 0·017 mm. at about the middle of its length. It tapers gradually towards both extremities, reaching a width of about 0·005 mm. at the head end and 0·008 mm. at the anus.

The tail exhibits the undulating appendix which is characteristic of the larvæ of this group of lungworms. It also shews a short pointed process which projects from the dorsal side of the tail and at the base of the undulating appendix (Fig. 3). The tail measures 0·03 mm. in length and the appendix 0·01mm.

The genital rudiment is situated about 0·12 mm. from the tip of the tail.

Owing to the small size of the worm it is difficult, even under an oil-immersion lens to make out many details of the structures in the œsophageal region and in the head end. The amphids are faintly indicated as two short ducts with lateral openings situated close to the anterior end. The œsophagus appears to be glandular, at least in the posterior half, and the lumen is slightly thickened in the anterior region giving the appearance of two fairly well defined rods when viewed in optical section. In one or two instances the ventral œsophageal (salivary) glands were seen to open into the lumen of the œsophagus in the region of the anterior bulb.

The lateral lines are very prominent in these larvæ and have the appearance of two well developed external ridges extending practically along the whole length of the body.

Biology.

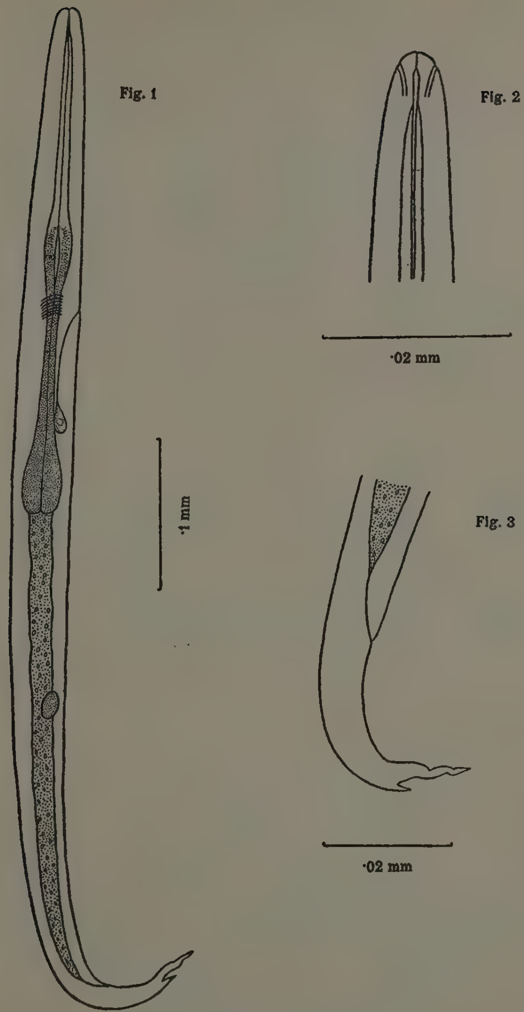
Effect of heat.—The reactions of the larvæ to heat were tested by means of an electrically heated stage placed upon the microscope. On this stage a slide was placed and a drop of water containing larvæ in suspension was added. The reaction of the larvæ was then noted at

different temperatures up to 50° C. This experiment was repeated three times with the following results.

All the larvæ shewed normal activity between the temperatures of 17° C. and about 27° C., but after this they distinctly shewed less movement as the temperature rose to 30° C. Between 30° C. and 35° C., activity became still less and at the latter temperature only a few shewed any signs of movement. From 35° C. up to 40° C. they became tightly coiled up and all were quite still. The temperature was then raised to 50° C. without any apparent change in the condition of the larvæ. The current was then cut off immediately on reaching this latter temperature and the whole allowed to cool. On cooling down to 37° C. two larvæ shewed slight activity and at 25° C. six were moving. Later the majority of the larvæ revived which shews that a temperature of 50° C. for a very short time is not sufficient to kill the larvæ. In another experiment the larvæ were left for half an hour at 45° C. but this was not sufficient to kill them. They did not revive, however, after leaving them at 50° C. for half an hour and only one shewed any movement after an exposure of 15 minutes at this temperature. The result of this experiment shews that the larvæ are most active at a temperature in the neighbourhood of 25° C. and that an exposure of from 15 to 30 minutes at 50° C. is sufficient to kill them. Complete observations on the effect of lower temperatures on the larvæ were not carried out but they were seen to be quite active at 17° C.

In one experiment to test the effect of freezing, some larvæ were placed in water in a glass capsule and left exposed outside the laboratory during frosty weather. After being frozen overnight the larvæ, on thawing, shewed no activity at first but in about an hour several revived.

Desiccation.—The power of the larvæ to revive after desiccation was most marked. Several experiments were carried out in the laboratory to test this, and although the results were not quite uniform there was sufficient evidence to shew that the larvæ can withstand a considerable period of desiccation. The initial experiments with larvæ on glass slides shewed that they could revive, on adding water after being dried up for three days. Following this the larvæ were placed in a number of glass capsules and allowed to remain dry for varying periods. The



Larva of Muellerius capillaris.

Fig. 1.—Showing entire larva.
Fig. 2.—Anterior end under high magnification.
Fig. 3.—Posterior end under high magnification showing undulating appendix.

results in this instance shewed that a considerable number were alive after seven days, and even after two weeks' desiccation slight movement was seen in a few but the majority were beginning to disintegrate. The worms in the capsule which had been left dry for a week, and which shewed a considerable number of living larvæ when water was added were then subjected to desiccation each day. They were allowed to remain dry overnight and water was added each morning. In this instance it was found that all movement ceased after three days of this treatment. In a similar experiment, fresh larvæ, *i.e.*, larvæ which had not previously been subjected to a period of dryness, were allowed to dry up every day. In this case it was found that the larvæ did survive after the third day but no sign of life was seen after the fifth day.

The results therefore indicate that, under laboratory conditions, the larvæ can withstand drying for over a week when desiccation is carried out without interruption. By daily alternation of wet and dry conditions it was found that the larvæ died in about four days.

It is difficult to judge whether the larvæ would meet such drastic conditions of dryness in the field even during a long period of drought, since the herbage would tend to maintain a certain amount of moisture on the soil surface. Dry summer conditions would, however, probably cause a certain amount of mortality among larvæ left in an exposed position. One might conclude from the laboratory experiments that frequent variations in climatic conditions would prove more effective in killing off the larvæ.

Skin-penetration.—Using the technique described by Goodey (1922), an experiment was carried out to determine whether the larvæ were capable of penetrating skin. A portion of skin from the abdomen of of a mouse was stretched out on a cork ring which was then floated on saline heated to a temperature of about 38° C. Larvæ in suspension in water were placed on the skin and their movements were then watched under a binocular microscope. Several of the larvæ shewed a fair amount of activity, but their movements shewed no downward tendency to penetrate the skin. On the whole they were not quite so inactive as they were on the hot stage at this temperature. After the water had dried up the larvæ were allowed to remain for half an hour and when water was again added they renewed their activity. After leaving overnight

the larvæ were still found on the surface of the skin and none were found in the saline below the cork raft.

Effect of Aniline Dyes.—The larvæ were found to be quite active in Fuchsin and did not appear to take up the stain.

Thermotropism and Heliotropism.—The normal activity of the larvæ is not so great as one finds in the infective larvæ of many nematodes, particularly in those inhabiting the alimentary tract. Even when the larvæ of *M. capillaris* are most active they do not seem to be capable of making much progress in a liquid medium. Their reaction, therefore, to a hot needle brought to touch the under side of a glass slide is difficult to estimate. There is no progress made either towards, or away from, the source of heat. The same difficulty was experienced in the experiments carried out to test the reaction of the larvæ to light.

Longevity.—Complete tests on longevity have not been carried out. Larvæ kept in water in the laboratory have in some instances died out in two or three weeks. It seemed, however, that whenever the water containing the larvæ shewed a good deal of bacterial activity owing to fæcal matter being present, the medium became too foul for the larvæ to live for any length of time. In one experiment where the larvæ had been placed in fairly clean water a good number were alive six weeks later and a few after eight weeks. None were alive, however, after three months in this instance.

Comparison with the Larvæ of Ælurostrongylus abstrusus.

As already referred to, the life history of *Æ. abstrusus* has been shewn by Cameron (1927) to be indirect, the larval stage, which is morphologically similar to the one described in this paper, requiring the intervention of an intermediate host before reaching the infective stage. The biology of the larvæ of these two species, although similar in morphology, shews some points of difference. In its reaction to heat, Cameron has shewn that the larva of *Æ. abstrusus* tends to increase its activity up to 37° C., and that afterwards the undulatory movement changes to a sharper lashing movement. In the larva of *M. capillaris* it has been shewn above that activity becomes reduced at a much lower temperature and that coiling takes place before reaching 37° C. In *Æ. abstrusus* coiling is not observed until 40° C. is reached.

Neither of the larvæ seem to be capable of penetrating the skin when tested by means of the Floating Raft technique, and both shew a similar reaction when brought into contact with aniline dyes. The larva of *M. capillaris* is, however, capable of remaining alive in the free state much longer than that of *Æ. abstrusus*, and coupled with this is the fact that it can withstand considerable periods of desiccation. On the whole, therefore, the former is more capable of resisting adverse conditions in the free state than the larva of *Æ. abstrusus*.

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The Species of *Enterobius* Leach, in Primates.

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The vast majority of the helminthic parasites of man are represented among those animals which are in close contact with man, either by the same species or at least, by closely related forms. A very noticeable exception is *Enterobius vermicularis*. Possibly owing to its special life-history, it has become highly specialised for its human host and to find its closest relations, it is necessary to examine the forms found in primates.

The object of this paper is accordingly to systematise our knowledge of the oxyurid worms found in this group.

As *Enterobius vermicularis* is the most important of these, it has been found desirable to briefly re-describe its anatomy, followed by that of the related species, in apes, in monkeys and other primates.

Enterobius vermicularis.

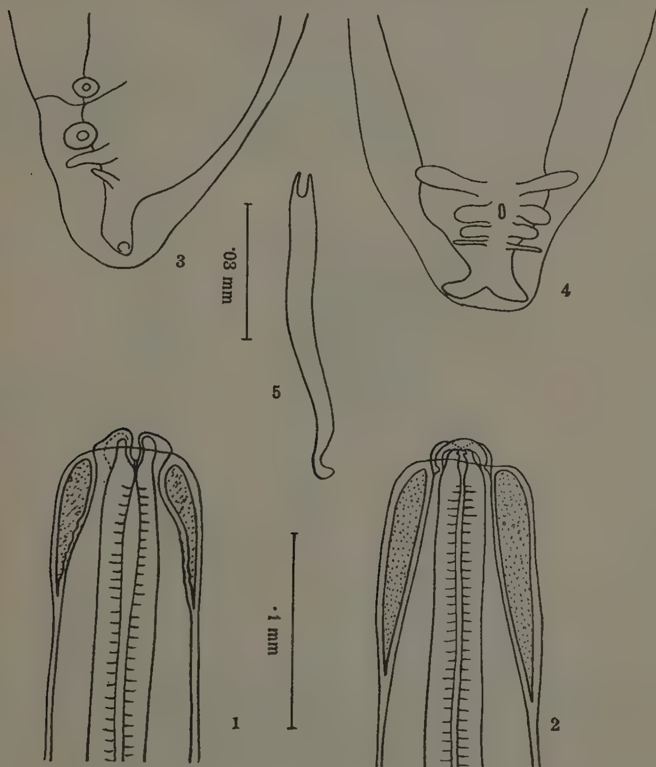
The cuticle is transversely striated and, narrow longitudinal alæ on the lateral lines, run from just behind the head almost to the tail. There is a distinct cuticular inflation in the head region which appears wider laterally than dorsally and ventrally, due to the thickening of the body (Figs. 1 and 2). The swelling is longer laterally than on the dorsal and ventral aspects. The œsophagus consists of an elongated thin anterior portion joined by a very narrow commissure to a posterior portion. The anterior portion has almost parallel margins but swells slightly in its

posterior half. The posterior " bulb " is longer than broad as a rule and contains a valvular apparatus. The anterior end of the œsophagus terminates in three lips, of which the dorsal is the larger. The pulp of these lips is a direct continuation of the œsophagus and is surrounded by an expansion of the body substance. The lips extend anterior to the cuticular expansion. There are no teeth present but the lips have small depressions on their inner margins which give the appearance of apical teeth when viewed laterally. The cephalic papillæ are relatively small : there are two present on the dorsal lip, and a larger lateral and a smaller ventral papilla on each of the ventral lips (Fig. 6). The intestine is normal. In the female it terminates in a fairly long straight rectum. At the junction of intestine and rectum are the usual three rectal " glands." The nerve ring is situated just behind the cephalic swelling. There are no cervical papillæ. The excretory pore is situated about the level of the posterior bulb of the œsophagus in young females and just behind this, in older forms.

The female (Fig. 7) varies in length from 2·5 mm. in young specimens to 12 mm. in gravid specimens. The maximum breadth is 0·6 mm. The vulva is situated at the junction of anterior and middle third and the anus about the junction of the fourth and posterior fifth. The female genitalia is double. The ovejector is divided into two portions—a short muscular portion with a relatively narrow lumen next to the vulva and a longer, very distendable thin-walled portion connecting the first with the uteri. This portion functions as a seminal reservoir in the young female : in gravid forms it becomes filled with eggs. The whole ovejector is often S-shaped and is directed posteriorly. The uteri leave the ovejector in opposite directions. The posterior uterus proceeds directly towards the tail while the anterior (which may double on itself) proceeds towards the head for a short distance and then turns and proceeds in a tail-ward direction. This is the appearance as seen in a young female with few ova. As the worm becomes gravid, the uteri become distended with ova until the whole body is filled with them (between the œsophagus and the anus—and sometimes posterior to the anus). The posterior uterus develops earlier and to a greater extent than the anterior uterus.

The male (Fig. 8) is about 3 mm. long and 0·2 mm. in width. The tail is directed ventrally and is truncated. The caudal papillæ, of which there are 5 pairs, support lateral alæ. The anterior pair of papillæ

(Figs. 3 and 4) are stout and club-shaped. These are followed by two shorter, stout pairs and by a third very slender pair. The terminal pair are stout, pointed and curved outwards. None of the caudal papillæ



Enterobius vermicularis.

Fig. 1.—Head, lateral aspect. Fig. 2.—Head, dorsal aspect. Fig. 3.—Tail of Male, lateral aspect. Fig. 4.—Tail of Male, ventral aspect. Fig. 5.—Chitinised portion of spicule.

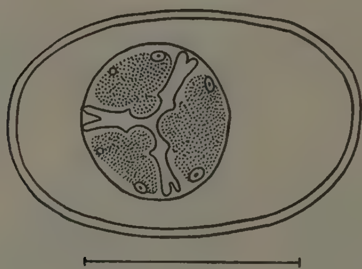
are directed posteriorly.

There is a single spicule, about 0·07 mm. long (Fig. 5). It has an undulating outline and tapers very gradually towards the blunt tip which

is curved ventrally to form a distinct hook. It has a depression anteriorly to which is attached a more or less oval basal portion. This is about two-thirds of the length of the spicule and about twice to three times its breadth and has a more fibrous structure. There is no accessory piece.

ENTEROBIUS ANTHROPOPITHECI (GEDOELST, 1916).

Only females of this species were found; they measured 4 to 5·5 mm. long by 0·326 to 0·360 mm. wide. The body is an elongated spindle, the anterior extremity being terminated by a vesicular swelling, while



Enterobius vermicularis.

Fig. 6.—Head of young female, viewed from anterior aspect.

the posterior is subulate. The cuticular striations, which are absent from the head, vary in distance. The cephalic swelling, which is formed by the bulging of the cuticle, is globular in form, measuring 0·12 mm. in diameter and 0·08 to 0·095 mm. long. It is sharply demarked from the body. Anteriorly there are three prominent lips which may, however, be withdrawn to lie at the base of a capsule formed by the cephalic swelling. The mouth is triangular and gives access to an œsophagus which is $\frac{1}{5\cdot75}$ of the body length. The first portion is long and narrow (0·04 mm.) but swells posteriorly until it is 0·072 mm. wide. It is connected by a short band (0·01 mm. long and 0·032 mm. wide) with the posterior bulb which is 0·12 mm. in diameter. The nerve ring is at the level of the first and second fifths of the œsophagus, and the excretory

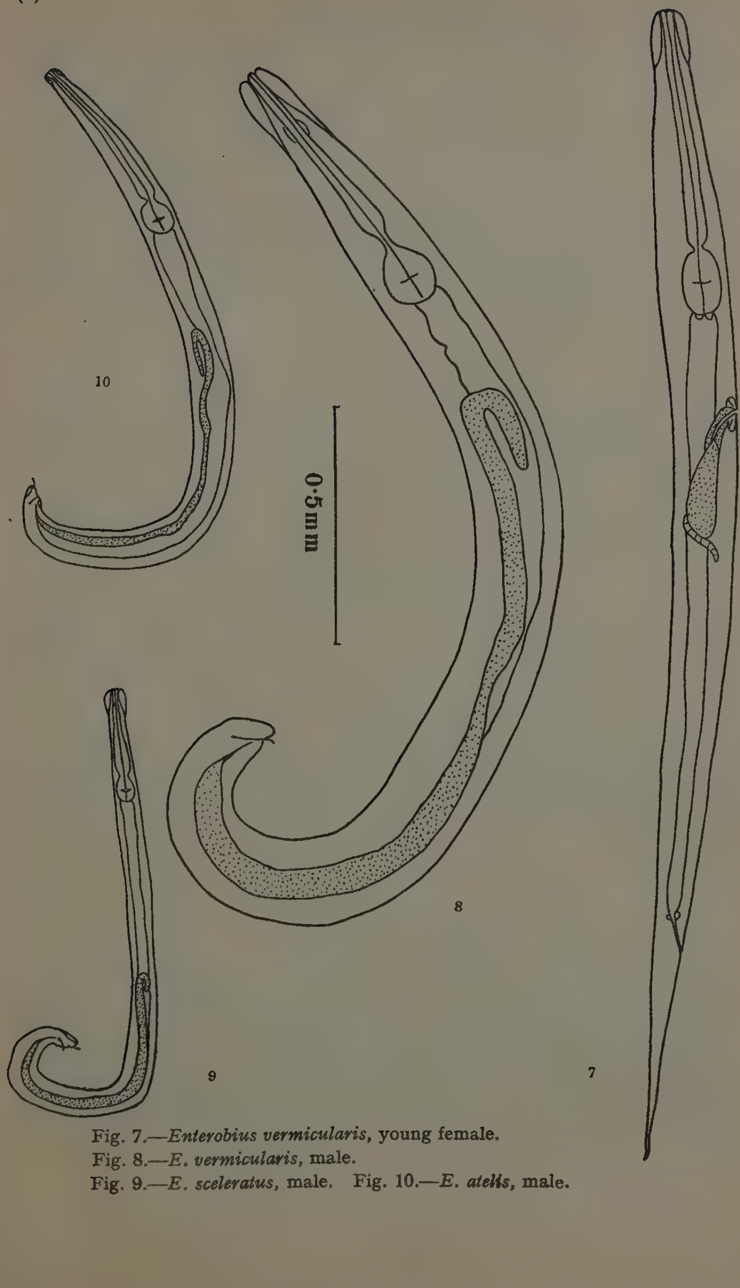


Fig. 7.—*Enterobius vermicularis*, young female.
Fig. 8.—*E. vermicularis*, male.
Fig. 9.—*E. sceleratus*, male. Fig. 10.—*E. atelis*, male.

pore at the level of the junction of œsophagus and intestine. The rectum is $0 \cdot 175$ mm. long and the anus is $1 \cdot 4$ to $1 \cdot 5$ mm. from the tip of the tail. The vulva divides the body in the ratio of $1 : 2 \cdot 35$—$2 \cdot 85$. The ovejector is an elongated cylinder and is curved posteriorly to meet two opposed uteri. The posterior uterus is prolonged posteriorly to turn anteriorly. Its ovarian tubule originates in the anterior part of the body. The anterior uterus, after a short forward path curves posteriorly, to curve again and proceed anteriorly. The ova are ellipsoidal, a symmetrical with a thin shell, and measure 70μ by 32μ. This species has been recorded from the Chimpanzee.

ENTEROBIUS FŒCUNDUS (V. LINST., 1879).

The mouth shows two small, slightly projecting, semi-spherical lips, behind which, in the submedian line, is a papilla. There are no lateral membrances: the cuticle is striated. The œsophagus, which measures $\frac{1}{6 \cdot 4}$ of the body length, terminates in an olive-shaped bulb, without any teeth, immediately in front of which the œsophagus is constricted.

The male is $1 \cdot 8$ mm. long and $0 \cdot 18$ mm. broad, with a tail bent in the form of a semi-circle. The tail is $\frac{1}{25}$ of the body length. It contracts suddenly and terminates in a straight portion which is bifurcated ventrally. There is a single pair of small post-anal papillæ. The almost straight spicule is $0 \cdot 052$ mm. long and ends roundly.

The female is $6 \cdot 5$ mm. long and $0 \cdot 66$ mm. broad and terminates in a long fine pointed tail which is $\frac{1}{8}$ of the body length. The mature female becomes filled with eggs, which causes all the organs—except the œsophagus—to become atrophied. The ova are 56μ by 26μ. This species occurs in the Orang.

ENTEROBIUS SIMIÆ (MACCALLUM, 1921).

This form has been reported by MacCallum from the Orang. It is 6 mm. long by $0 \cdot 3$ mm. wide. It is small and of a very delicate shape, nearly white in colour and almost transparent. The mouth is terminal and surrounded by small papillæ, not at all armed: it communicates with the pharynx by means of a long œsophagus, which terminates in a globular portion or valve. "The vagina is at about the beginning of the posterior third of the worm. Eggs are small, yellow and oval, tail very

pointed and the anus opens some distance from the tip, in a slit like opening." "Both sexes are much alike in appearance."

The last statement seems improbable from our knowledge of related species of Oxyurids. It is possible that this form is identical with the previous species (*E. fœcundus*).

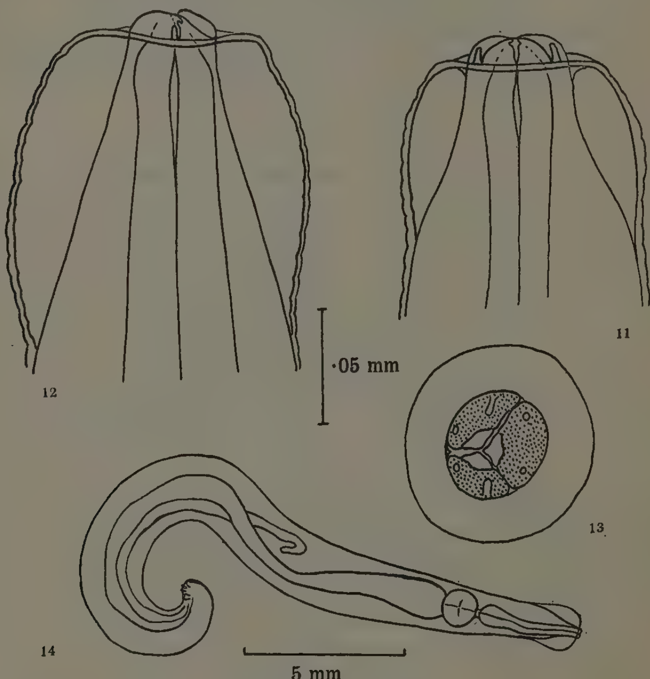
Enterobius bipapillatus (Gedoelst, 1916).

The body, cylindricoid in form, attentuates anteriorly until 0·125 to 0·175 mm. from the extremity, where the cuticle expands to a diameter of 0·09 to 0·13 mm. This swelling commences suddenly at the base of the lips but insensibly runs into the body posteriorly. Lateral alæ arise towards the middle of the œsophageal region and run to near the tail where they gradually die away. The cuticle is striated, the striations being 9—10μ apart; this striation is continued on to the cephalic swelling. The mouth is limited by three sub-globular lips, 10μ high. It has on each side, a lateral cephalic papilla. The œsophagus consists of an elongated portion swollen posteriorly and an œsophageal bulb, slightly longer than wide, connected to the anterior portion by a short narrow neck. The nerve ring is at the anterior third of the œsophagus.

The females measure from 4·3 to 7·3 mm. long and from 0·48 to 0·64 mm. thick. The œsophagus, 0·9 mm. long, represents $\frac{1}{5}$ to $\frac{1}{8}$ of the body length. The tail is long and pointed and is about $\frac{1}{5}$ of the total length. The vulva is in the anterior third and has two slightly salient lips. In the young female the genital system consists of a short cylindrical vestibule about 160μ long, and 0·055 to 0·06 mm. wide, which is continued posteriorly as an unpaired fusiform tube about 0·5 mm. long. At this point it bifurcates. The uteri diverge; the anterior runs to within 0·4 mm. of the origin of the intestine; the posterior runs for about 0·65 mm. when it turns and runs anteriorly to terminate at the same level as the anterior. In the gravid female the whole apparatus is transformed into a fusiform sac full of eggs, occupying the whole body from the œsophageal bulb to the anus. Only the ovejector remains. The intestine is pressed against the wall of the body and the striations are extended to a distance of 42μ from each other. The eggs, of the typical Enterobius shape, measure 64 to 68μ by 32μ.

The male is 2·15 to 2·50 mm. long and about 0·245 mm. thick. The

œsophagus is about a quarter of the body length. The posterior extermity is slightly attenuated and is terminated by a short obtuse tail; the cloaca is subterminal. There are 4 pairs of caudal papillæ, the anterior pair being at the side of the cloacal opening while the first and fourth pair are strongly developed and relatively salient. They raise the cuticle to form a small bursa. There is a single spicule, 80μ long with a round swollen head (12μ wide) and a fine, gently bent tip.



Enterobius bipapillatus.

Fig. 11.—Ventral view of head. Fig. 12.—Lateral view. Fig. 13.—Anterior view. Fig. 14.—Male.

This species was reported by Gedoelst from an undetermined monkey from Central Africa.

I have been able to examine about a dozen females and a single male of what appear to be this species from *Cercopithecus sabaeus* from West

Africa. The females were slightly smaller than the type, measuring about 4·1 to 5·2 mm. long by 0·3 to 0·4 mm. wide. The œsophagus is about one-sixth of the body length and the tail is one-fifth. The vulva is situated just posterior to the junction of the anterior and middle thirds of the body. There are three sub-globular lips which project beyond the limits of the cephalic swelling. The dorsal lip (Figs. 11–13), is the largest and has a more pointed apex than the two ventral lips. In all, the apex is cuticularised and marked off from the pulp by a small constriction. Each lips carries two papillæ, but the lateral papillæ of the ventral lips are very much larger than the others.

The vulva is situated just posterior to the junction of the anterior and second third of the body. The genitalia agrees with Gedoelst's description.

The single male (Fig. 14) is 2·25 mm. long and 0·21 mm. broad. The œsophagus is rather longer than described by Gedoelst, being about one-fifth of the body length. The posterior extremity is truncated. Unfortunately it was slightly damaged and I am unable to state whether there are 4 or 5 pairs of papillæ present. The spicule also was broken.

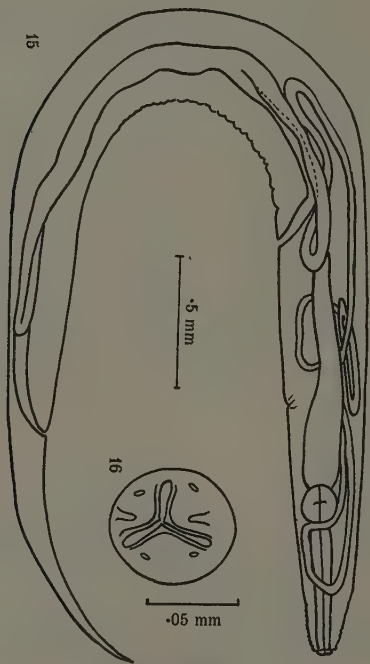
The differences between this form and Gedoelst's description are so slight that there is little doubt that they are identical.

Enterobius Pitheci sp. nov.

In 1923, Baylis reported on some specimens from *Pithecus entellus* and referred these to Gedoelst's species, in spite of some slight discrepancies in measurements, etc. The males had an extra pair of caudal papillæ, in a lateral position at the level of the two small ventral pairs shown by Gedoelst. The spicule was only about 60μ long, instead of 80μ. *P. entellus* is an Indian monkey, whilst Gedoelst's was African. Recently, I have been able to examine some specimens from *P. aygula* from Assam. Unfortunately, only females were found (Fig. 15).

They measure about 7·3 mm. long and have a maximum breadth of 0·5 mm. Small lateral alæ are present. The cuticle is markedly striated in the anterior region of the body but posteriorly the striations almost disappear. There are three conspicuous lips, the dorsal slightly overhanging the mouth opening. All three project beyond the anterior

limits of the cuticle. The dorsal lip carries two small papillæ while each ventral has a small papilla in a ventral position and a large fleshy papilla in a lateral position. The apex of each lips is prolonged inwards to form a minute tooth-like structure (Figs. 16—18). The œsophagus measures from 0·6 to 0·75 mm. long (including the bulb) and has a

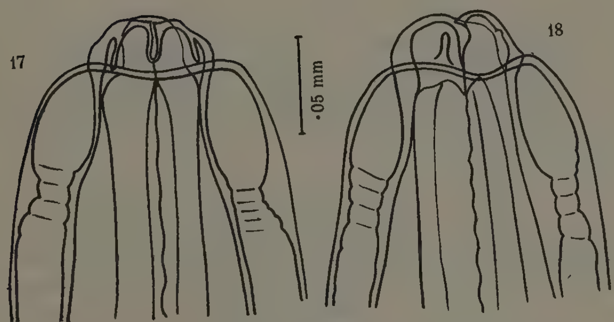


Enterobius pitheci.

Fig. 15.—Young female. Fig. 16.—Anterior view of Head.

maximum width of 0·075 mm. The diameter of the bulb is about 0·125 mm. The complete œsophagus is therefore about one-tenth to one-twelfth of the body length. The female genitalia corresponds

with Gedoelst's description. The vulva is at the junction of the anterior and second third. The anterior part of the vagina is modified to form an ovejector ; the whole is directed backwards and is joined to two divergent uteri. Both ovarian tubules are in the anterior part of the body and actually have coils in the œsophageal region. The tail is long and pointed and is about one-sixth to one-fifth of the body length. The ova are typical and measure about 50μ by 25μ.



Enterobius pitheci.

Fig. 17.—Ventral view of Head. Fig. 18.—Lateral view of Head.

There are several minor differences between this form and that described by Gedoelst, and it is, accordingly, difficult to identify it with *E.bipapillatus*. These differences in morphology (structure of head, length of œsophagus, size of eggs) together with the generic differences of the hosts and the distribution justify one, as a tentative measure, in placing it in a new species. Accordingly, the name *E. pitheci* is proposed for it. It is possible that the forms referred by Baylis to *E. bipapillatus* should really belong to this new species.

Enterobius microon (v. Linst., 1907).

The cuticle is transversely striated. The cephalic extremity has a vesicular dilitation and three rounded lips. The œsophagus is very long ; 0·61 mm. in the male and 0·73 mm. in the female. The œsophageal bulb is separated from the remainder by a constriction. The excretory pore is under the œsophagus (0·93 mm. from the anterior end in the female).

The female is 4·43 mm. long by 0·31 mm. wide. The tail is 1·10mm. long and finely subulate. The vulva is in the anterior part of the body dividing in 9 : 25. The vagina is directed posteriorly and the uteri are divergent. The ova measure 42μ by 23μ.

The male is 1·42 mm. long by 0·13 mm. wide. There is a straight spicule, 49μ long. The tail has a vesicular dilation, dorsally and ventrally with a pair of conical papillæ.

This species has been reported only from *Aotus* (=*Nyctipithecus*) *trivirgatus* from Brazil.

Enterobius trypanuris (Vevers, 1923).

This species (fide Vevers) is a small fusiform worm with the cuticle transversely striated at the extreme anterior end, which is smooth and slightly expanded. The mouth has two inconspicuous lips. The œsophagus is of the usual oxyurid type.

The female is 6·7 mm. long by 0·6 mm. in diameter. The œsophagus is 0·9 mm. long and the bulb 0·15 mm. in diameter. The tail is gently tapering and ends in a blunt point. The distance of the anus from the tail is 0·775 mm. The vulva is at the junction of the middle and anterior thirds. The ova measures 45 by 30μ.

The male is 2 mm. long by 0·15 mm. in diameter. The œsophagus is 0·3 mm. long and 0·05 mm. broad with a bulb 0·075μ in diameter. The posterior end terminates in a rounded extremity carrying a sharp spike 0·012 mm. in diameter. Caudal alæ are present and there are five pairs of papillæ—two pre-and three post-anal. The spicule is 0·07mm. long and is stated to have a chitinous annular accessory piece.

Baylis and Daubney point out in their Synopsis that the accessory piece as depicted by Vevers would prevent the extrusion of the spicule.

An examination of the females showed that there are three lips present as in the other species described in this paper. Another point which is worthy of attention is that the tail of the female is practically always bent to form a " pot-hook " and compared with the other species under review, is remarkably short.

This species has only been found in *Pithecia monachus* from Guiana.

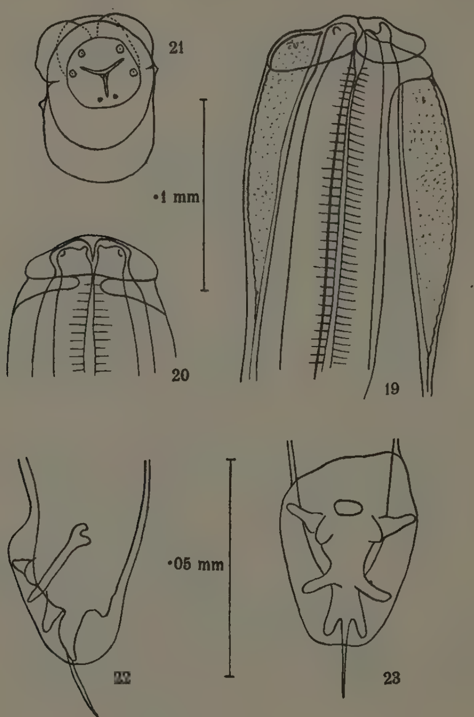
Enterobius sceleratus Travassos, 1925.

A considerable number of females in various stages of development and several males of this species were collected by the writer from the cæca of several new world monkeys (*Saimiri sciurea*). The cuticle is transversely striated and lateral cuticular alæ are present. The cephalic cuticle is arranged in a most peculiar manner and is always swollen, sometimes considerably, the expansion being abruptly separated from the unexpanded portion. It can be divided into three distinct portions. The first, or circum-oral portion (Figs. 19 to 21) is cap-shaped and completely surrounds the mouth. It has two lateral constrictions and the ventral is much larger than the dorsal portion. Posterior to this is a second swelling which, mainly ventral, projects on to the lateral and dorso-lateral regions, in the anterior part of the body. These dorsal extensions approach the mid-dorsal line but do not meet. The third part is dorsal and extends from the dorsal half of the cicum-oral swelling for about the same distance down the neck as does the second or latero-ventral expansion. These swellings are filled with a very finely granular material. The mouth opening is tri-radiate with a large dorsal and two smaller latero-ventral lips: the lips have two papillæ each. The two ventral lips each have a minute internal tooth (Fig. 21). The pulp, which is a direct continuation of the muscular œsophagus, does not completely fill the lips and in the dorsal lip it has a dorsal extension.

The œsophagus has a slight swelling at the end of the anterior portion which (in the gravid female) is about 0·5 mm. long and 0·08 mm. broad. The posterior bulb is nearly spherical with a diameter of 0·1 mm. The nerve ring is just posterior to the cephalic swelling and the excretory pore just posterior to the œsophagus.

The gravid female is about 6·5 mm. long and about 0·3 mm. in

maximum thickness. The vulva is about the level of the junction of the anterior and second third of the body and the anus is about 1·5 mm. from the tail which narrows gradually from this point to the sharp tip.



Enterobius sceleratus.

Fig. 19.—Lateral view of head. Fig. 20.—Dorsal view. Fig. 21.—Terminal view. Fig. 22.—Tail of male, lateral view. Fig. 23.—Ventral view.

The vagina is a short broad posteriorly directed tube which divides to form the two uteri. These are continued as the ovaries—one of which turns just posterior to the vulva and ends posteriorly to the anus: the other turns just behind the œsophagus and ends just posterior to the vulva.

The ova are typical and measure about 55μ by 27μ. They are very faintly "pitted."

The male (Fig. 9) is about 1·25 mm. long by 0·05 mm. broad. It has a bluntly truncated tail which is curved ventrally. The body substance does not completely fill the tail in the dorsal region and there is a considerable space at this point (Figs. 22 and 23). A broad cuticular expansion is present on either side of the caudal region. The end of the body projects through this expansion to form a sharp terminal spike 0·015 mm. long. There are four pairs of caudal papillæ. The anterior pair is finger-like and is directed antero-laterally. The second pair is sessile and situated at the base of the first pair and just posterior to the ano-genital opening. The third and fourth pair are posterior. The third pair is elongated and directed laterally and the fourth pair, from between which the caudal spike rises, is directed posteriorly. There is a single blunt straight spicule 0·02 mm. to 0·03 mm. long with a bifurcated anterior portion. There is no accessory piece.

The type host of this parasite is *Saimiri sciurea* from South America but I have also found it in *S. örstedii* from Central America.

Enterobius minutus (Sch., 1866).

This parasite was described by Schneider from *Alouatta seniculus* and *Ateles paniscus* as follows :—

Female, 8·5 mm., male, 3 mm. long. Four distinct papillæ on the head. Lateral membrances present. Vulva shortly behind end of œsophagus. The vagina, which runs posteriorly, is divided and the branches of the uterus diverge. Egg shell punctate. Tail of male spirally coiled and blunt with a short thread-like spike. Two papillæ. One at the level of anus. In *Mycetes seniculus* and *Ateles paniscus*, Brazil.

Schneider states that this species differs from *O. vermicularis* in shape of the bursa, length of the œsophagus (1·7 mm. in *O. minuta* and 1 mm. in (*O. vermicularis*) and in the shape of the cross section of the lateral line His text-figures shows three pairs of papillæ and a single pointed spicule with a rounded base.

In 1925, v. Thiel reported from the first host a species which he called

S. bonnei but which Travassos states is identical with Schneider's. Only females were found. They measured 8·5 mm. long by 0·48 mm. wide. The cuticle is striated, the striations being most conspicuous in the middle region of the body. Lateral cuticular expansions to the head region are found and they are prolonged to form narrow lateral crests. There are three large lips. The buccal cavity is short. The œsophagus is 1·9 mm. long with a posterior bulb 0·14 mm. in diameter. The uterus is large and voluminous and the vulva is in the anterior third of the body. There is a short muscular ovejector followed by a long non-muscular vagina. The ova, which measure 41·8μ by 28·8μ have a punctate surface due to pores.

He points out that, while it resembles *O. minuta* in dimensions of body and œsophagus, situation of vulva and double uterus, the female of that species has four cephalic papillæ which are absent from this and the shapes of the longitudinal crests are probably different.

Travassos has found in a number of specimens of *Alouatta caraya* a species which he considers is identical with the forms described by Schneider and by v. Thiel.

Female: 8 to 10 mm. by 0·4 to 0·5 mm. *Male:* 2 to 3 mm. by 0·1 mm. Posterior extremity elongated in female, truncated in male. Anterior extremity with three bilobed, much reduced lips. There is a vesicular dilitation of the cuticle resembling that seen in *E. vermicularis* but without transverse striations and with a median constriction. The œsophagus is very long and has a terminal part in contact with the bulb, slightly dilated and more transparent. It is about 1 mm. long in the female and 0·46 mm. to 0·71 mm. in the male. The œsophageal bulb, which is provided with the usual valvular mechanism, is 0·1 mm. in the female and 0·036 to 0·078 mm. in the male. The excretory pore opens a little above the œsophageal bulb; nerve ring is behind the cephalic swelling.

In the female, the vulva is situated in the anterior half of the body, slightly salient and with a muscular ovejector situated behind the vagina. The ovejector opens into a large vestibule which functions as a seminal receptacle at first and later for the deposition of ova. From the vestibule runs a narrow canal which bifurcates to form the divergent uteri. The tail is about 1·7 mm. long.

The male has the tail truncated and carries a very delicate terminal

spine 10μ to 11μ long. The lateral alæ are supported by three pairs of papillæ—a large pre-anal pair, a very slender adanal pair and a pair of post-anal papillæ, a little smaller than the pre-anal. The caudal alæ have a depression at the level of the adanal papillæ. The spicule has a sharp distal end and the proximal end dilated to form a spatula with a posterior notch. The spicule is 40μ to 55μ with a diameter, in the region of the dilation, of 8μ. Anus 10μ to 12μ from the posterior extremity (disregarding the spine).

Enterobius atelis sp. nov.

From several different species of the genus *Ateles* I have examined an oxyurid which appears to be closely related to, but not identical with, the forms described by v. Thiel and by Travassos. The cuticle is markedly striated and has two very narrow lateral cuticular crests. It is only slightly swollen anteriorly but as the body abruptly narrows in the head region, there is actually a considerable cephalic vesicle (Figs. 24 and 25). The lips project beyond the cuticular inflation. There is a large dorsal lip which overhangs the mouth cavity; but the ventral lips are fused together externally with the result that the mouth-opening is a transverse slit (Fig. 26). At the base of the oral cavity are two ventral œsophageal teeth. The usual six circum-oral papillæ are present, but all are very small, although the lateral pair are slightly more conspicuous than the sub-dorsal and sub-ventral. The œsophagus is of the typical oxyurid type. The first portion is swollen posteriorly and measures, in a gravid female, about 0·75 mm. to 0·85 mm. in length and 0·09 mm. in diameter at the swelling. It is connected by a narrow commissure to the posterior bulb which has a diameter of about 0·12 mm. The œsophagus is about one-sixth to one-seventh of the body length. The nerve ring is situated about the junction of the anterior and second quarter, while the excretory pore is just posterior to the junction of intestine and œsophagus.

The female measures from 3·25 mm. in young specimens to a maximum of 6 mm. in gravid specimens. The maximum breadth of gravid females is 0·4 mm. The vulva is situated about the junction of the second and third fifth and the anus about the junction of the fourth and last fifth

of the body. These proportions appear to be constant for the various ages. The tail is long and pointed and tapers uniformly from the anal region to the tip. The genital system is similar to that in *E. vermicularis*. The ova are of the typical bean-shaped form and measure about 40μ by 20μ. Under a $\frac{1}{12}$-in. objective they are seen to be faintly "pitted."

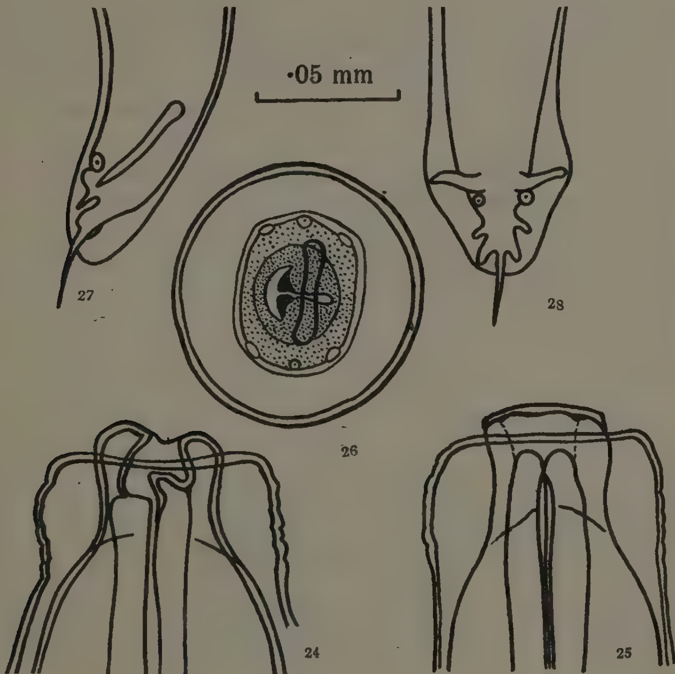
The male (Fig. 10) measures about 1·5 mm. long by 0·1 mm. broad. The tail is truncated and directed ventrally. The body wall is retracted from the cuticle on the dorsal aspect of the tail, and it projects through the cuticle ventrally to form a sharp spike about 0·025 mm. long (Figs. 27 and 28). There are four pairs of papillæ. The first pair is long and finger-like and reaches the edge of the cuticular expansion. The second pair is short and rounded. The third pair is small and narrow and does not reach the edge of the cuticle. The fourth pair is stout but elongated and directed posteriorly. The terminal spike rises from between this last pair. The spicule is single, short and pointed with a constriction towards the anterior end. It is about 0·042 mm. long. There is no accessory piece present.

This form occurs in *Ateles paniscus*, *A. ater* and *A. grisescens* all from South America.

This form is obviously closely related to *E. minutus* but differs from the descriptions of it in a number of particulars; including smaller size structure of head, presence of buccal teeth, tail of male and appearance of eggs. The eggs, although pitted, appear smooth-shelled with the highest dry powers of the microscope.

Schneider reported *E. minutus* from two species of South American monkeys: *Alouatta* and *Ateles*. Travassos finds that the form he has described as *E. minutus* is a common parasite of *Alouatta* in Brazil, and apparently no other species have been found in this genus. I find that the forms occurring in *Ateles* differ from Travassos' description and it is probable that each genus of monkey has its own particular species of parasite. Accordingly it seems legitimate to conclude that Schneider confused two species under the one name. His description is sufficiently general to apply to either; but he mentions that the eggs are "pitted." These pittings are practically invisible in the forms from *Ateles* and it is doubtful if he could see them with the optical equipment of his time. Moreover, he cites *Alouatta* first in mentioning the hosts and Travassos has described the forms in that genus of monkey under Schneider's name.

Accordingly, *E. minutus* would be the correct name for the oxyurid from that genus of monkey; while the species from *Ateles* would be named *E. atelis*.



Enterobius atelis.

Fig. 24.—Lateral view of head. Fig. 25.—Ventral view of head. Fig. 26.—Terminal view of head, shewing mouth opening, circum-oral papillæ and œsophageal teeth. Fig. 27.—Lateral view of tail of male. Fig. 28.—Ventral view of tail.

Enterobius nycticebi Baylis, 1928.

This is a considerably smaller species than the type which it otherwise closely resembles. The male is 2·2 to 2·4 mm. by 0·22 to 0·25 mm., and the female 4·5 to 6 mm. by 0·4 mm. The cuticular striations are

finer anteriorly than posteriorly, being about 0·0025 to 0·005 mm. apart in the male and 0·005 to 0·01 mm. in the female. The œsophagus (including bulb) is 0·4 to 0·45 mm. in the male and 0·6 to 0·65 mm. in the female. The nerve ring is 0·12 mm. in the male and 0·17 mm. in the female; the excretory pore is 0·7 mm. in the male and 0·95 to 1·05 mm. in the female from the anterior end. The bulb of the œsophagus is 0·12 to 0·14 mm. by 0/1 to 0·12 mm. in the male and 0·16 to 0·17 mm. by 0·14 to 0·15 mm. in the female. The anterior portion is gradually swollen behind then diminishes suddenly to form a very narrow neck before joining the bulb.

Female: The tail is long and tapering (1 to 1·3 mm.); the vulva is 1·5 to 1·75 mm. from anterior end (1 : 3); the ova 0·0875 by 0·0373 mm.

Male: The tail is rounded and very short (0·03 to 0·047 mm., including a minute tail spike measuring up to 0·01 mm. long). The spicule is slender and tapers to a fine point without a terminal hook. It is 0·1 mm. long and consists of a basal portion (bifid when viewed dorso-ventrally) and a darker tubular portion (0·088 mm. long). The papillæ are similar to *E. bipapillatus* (see Baylis, 1923) (*e.g.*, four pairs post-anal and one large pre-anal pair). The fifth post-anal pair seen in *E. vermicularis* was not seen.

This species differs from *E. bipapillatus* in having a shorter œsophagus, in the position of the vulva (latter 1 : 2), in the longer spicule, in the larger ova, and in the presence of a tail spike. It differs from *E. trypanuris* in above features (except last).

Baylis records this species from *Nycticebus borneanus* from Sarawak: I have found what appears to be this species in *N. coucang* from Malay. Only females were present and their state of preservation prevents any amplification of Baylis's description.

Conclusion.

The genus *Enterobius* is a peculiar one among the parasites of primates in that its life history tends to make it a parasite of the individual. Its eggs do not tend to be broadcast as do those of the other helminths and consequently there must be a tendency for any one species of parasite to restrict itself to the same species of host. The examination of the forms described in this paper suggests that one species restricts itself

to one genus of host rather than to one species; in other words the evolution of the parasite is slower than that of the primate. It would seem legitimate to assume, to some extent at least, that the parasite has evolved with the host. If one assume the existence of a *pre-enterobius* form in the *pre-simian* host, then the modifications of the parasite should accompany the generic differences of the host. One would expect to find forms most closely related to the human parasite in apes, while those in old world monkeys would be closer than *E. vermicularis* than those in new world monkeys and the lories but not so close as in apes. This actually does seem to be the case although many species are inadequately described and many other species of monkeys have to be examined before the series will be sufficiently extensive to justify any results of value to anthropology.

ORDER: PRIMATES.

Hominidæ ...	*Homo sapiens*	Cosmopolitan ...	*E. vermicularis*
Pongidæ	*Pan satyrus* ... ... (Chimpanzee)	Tropical Africa ...	*E. anthropitheci.*
	Pongo pygmæus (Orang)	Oceania	*E. fœcundus* (=*simiæ* ?)
Cercopithecidæ ...	*Pithecus entellus* ...	India	*E. bipapillatus ?*
	P. aygula	Assam	*E. pitheci.*
	Monkey, sp. inq. ...	Africa	*E. bipapillatus.*
	Cercopithecus sabaeus ...	,, ...	,,
Cebidæ	*Aotus trivirgatus* ...	Brazil	*E. microon.*
	Pithecia monachus ...	Guiana	*E. trypanuris.*
	Saimiri sciurea ...	Northern S. America	*E. sceleratus.*
	S. örstedii	C. America ...	,,
	Ateles paniscus... ...	S. America ...	*E. atelis.*
	A. ater	,, ...	,,
	A. grisescens	,, ...	,,
	Alouatta seniculus ...	,, ...	*E. minutus.*
	A. caraya	,, ...	,,
Lorisidæ	*Nycticebus borneanus*...	Borneo	*E. nycticebi.*
	N. coucang.	Malay	,,

In the list of hosts, I have adhered to the nomenclature adopted by the London Zoological Society in its "List of the Vertebrated Animals" (Volume I), 1929.

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The Stem Eelworm, *Tylenchus dipsaci* (Kühn, 1858): Observations on its attacks on Potatoes and Mangolds with a Host-list of plants parasitized by it.

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Tylenchus dipsaci on Potatoes.

(i.) *Introduction.*

The nematode has been known as a serious parasite of potatoes for a number of years. The disease produced by it was first described in Germany by Kühn (1888) under the name of Wurmfäule. Shortly after this Ritzema Bos (1892) confirmed and amplified Kühn's account and carried out an experimental investigation on the disease. The present writer Goodey (1923) gave a short résumé of this earlier work and an account of the occurrence of the disease on potatoes in the Holbeach area of Lincolnshire. Buckhurst (1925) also reported the results of certain successful pot experiments in which potatoes were infected by growing in soil containing diseased tuber material. Finally, an important paper by Quanjer (1927) has appeared in which he deals with the occurrence of the disease on potatoes at Wageningen, Holland, and shows that the physiological race of worms attacking potatoes there occurs naturally on certain wild plants in the pastures and is capable of attacking a wide range of host plants both cultivated and wild. He also discusses the mode of entry of the parasite into the host and the nature of its action on the plant tissues.

(ii.) *Field plot experiments at Winches Farm.*

In the autumn of 1926 an area of 1½ acres was marked off on an arable field at Winches Farm to be devoted to a series of experimental plots to carry crops suffering from various diseases due to the attacks of parasitic nematodes. This area was divided up into 15 small plots each 1/10th of an acre in size and measuring 200 ft. long by 21ft. 8⅛ ins. wide. Adjacent plots were separated by a path 2 ft. wide. One of these plots was selected for work on potatoes affected by *T. dipsaci*.

In March, 1927, about 10 cwts. of diseased potatoes, *King Edward* var., were put down to serve as a source of infection. Eight furrows were opened up down the length of the plot 2 ft. 6 ins. apart and about 9 ins. deep and the tubers were placed practically touching each other, in each furrow, the soil being finally drawn over them.

About the middle of April, 1927, the plot was planted with *King Edward* seed potatoes. The sets were dibbled in holes made on the rows already put down with diseased tubers. The soil in between the rows was cultivated and in due course ridging was carried out. On lifting the crop in the autumn it was found that large numbers of the tubers were attacked by *T. dipsaci*. The produce of the whole plot was clamped in a pit made at the west end of the plot. A dressing of 2 cart-loads of farmyard manure was applied and dug in in late autumn.

Early in 1928 the pit was opened and the tubers were returned to the plot in order to carry on the infection of *T. dipsaci*. Furrows were made as before and the potatoes were placed in these. There were eight rows in all 2 ft. 6 ins. apart. No tubers were put down on the last 20 ft. at the east end of the plot which was left as an area on which to test for any residual infection from the 1927 crop supposing the worms had remained in the soil.

Arrangements were made for a varieties trial to test the susceptibility of early, second-early and main-crop varieties to the parasite. The varieties were selected to represent some of the principal ones in commercial cultivation as follows :—*Earlies*, Epicure, Sharpe's Express, Duke of York. *Second-earlies*, Great Scot, British Queen. *Main-crop*, King Edward, Majestic, Arran Chief, Kerr's Pink, Up-to-date.

Except for the 20 ft. strip at the east end, the plot was divided into 30 small areas each measuring 12 ft. long by 10 ft. 9 ins. wide so that each of the ten varieties could be grown in triplicate. The distribution

of the varieties was arranged so as to give the widest scattering of ten varieties amongst thirty plots and the final lay-out is shown in the following plan.

EAST.

KING EDWARD.	
29 GREAT SCOT.	30 EPICURE.
27 SHARPE'S EXPRESS.	28 UP-TO-DATE.
25 KERR'S PINK.	26 MAJESTIC.
23 ARRAN CHIEF.	24 KING EDWARD.
21 DUKE OF YORK.	22 BRITISH QUEEN.
19 MAJESTIC.	20 ARRAN CHIEF.
17 UP-TO-DATE.	18 KERR'S PINK.
15 KING EDWARD.	16 SHARPE'S EXPRESS.
13 BRITISH QUEEN.	14 DUKE OF YORK.
11 ARRAN CHIEF.	12 GREAT SCOT.
9 EPICURE.	10 KING EDWARD.
7 UP-TO-DATE.	8 MAJESTIC.
5 BRITISH QUEEN.	6 EPICURE.
3 DUKE OF YORK.	4 SHARPE'S EXPRESS.
1 KERR'S PINK.	2 GREAT SCOT.

WEST.

Four rows of each variety were planted on each small plot parallel to the long axis, the rows being 2 ft. 6 ins. apart. The 20 ft. strip at the

east end received eight rows of King Edward in line with the rows throughout the whole plot. Planting was done on April 13th.

On most of the plots the plants looked fairly healthy. An exception must be noted in the case of Epicure which did not look vigorous on any of its three plots and was particularly weak looking on plot thirty. Cultivation between the rows was carried out by forking and by means of a "Monotrac" cultivator and some of the ridging was also done by the latter. A good number of the King Edward potatoes, returned to serve as the source of infection, grew and produced plants alongside the different varieties.

The crop was dug on October 8th and 9th and the tubers of each variety were bagged up separately on October 12th and brought down to the laboratory. The tubers of King Edward resulting from the returned 1927 crop were kept separate from each variety except in the case of the three plots devoted to that particular variety where they were taken up all together. The crop from the 20 ft. strip at the east end was also bagged up separately.

A detailed examination of the produce of each plot was next carried out. The number of tubers for each was counted and every tuber was carefully examined for the presence of disease or any sign of damage. Those showing disease or damage were set apart from the sound ones and were then further examined in detail for the presence of *T. dipsaci*. It is not a difficult matter, after a little practice, to recognise the external characteristics of those tubers definitely attacked by the parasite. There is frequently a splitting of the skin over the diseased, discoloured area. In the case of lightly affected tubers the skin may not have become shrunken and cracked and only a slight discolouration or mottling of the skin in a few spots may be noticed. Such lightly affected tubers are easily passed over and are difficult to recognise without careful scrutiny. It is quite probable that the disease is spread from place to place by means of such lightly affected tubers getting into stocks of seed potatoes. When a cut is made under the skin of a well-diseased tuber the tissues most frequently show a light brownish-white appearance and are mealy and soft to the touch. The cells making up the material easily separate from one another when touched with a needle or a scalpel. A lightly diseased tuber when cut may not show any discoloured cells but merely a small glistening white area in which the cells are very loosely held

together. In the case of every tuber suspected of attack by *T. dipsaci* a small portion of the affected tissues was examined under the microscope and the presence of the worms established.

Having counted the total number of tubers from each plot and determined the number of these affected by *T. dipsaci* it was a simple matter to estimate the percentage infection for each plot. The results are set out in the accompanying table.

Variety.	Plot No.	Total No. of tubers.	No. with *T. dipsaci*.	Percentage.	Average per cent. of three plots.
Epicure	6 19 30	106 235 124	7 29 16	6·6 12·34 12·9	10·61
Sharpe's Express	4 16 27	201 210 180	5 15 8	2·5 7·14 4·44	4·88
Duke of York ...	3 14 21	120 176 103	15 10 4	3·5 5·69 3·9	4·36
Great Scot ...	2 12 29	192 258 191	6 19 13	3·13 7·37 6·8	6·1
British Queen ...	5 13 22	182 250 200	2 25 17	1·1 10·0 8·5	6·5
King Edward ...	10 15 24	435 326 435	12 6 13	2·75 1·84 3·0	2·53
Majestic	8 19 26	235 204 140	7 3 5	3·0 1·47 3·57	2·68
Arran Chief ...	11 20 23	288 225 213	12 17 6	4·16 7·55 2·81	4·5
Kerr's Pink ...	1 18 25	187 204 237	1 10 14	0·53 4·9 5·9	3·77
Up-to-Date ...	7 17 28	331 274 320	11 7 13	3·3 2·55 4·06	3·3

The figures indicate that the infection was present throughout the plots and that all the varieties tested are susceptible to attack by *T. dipsaci*. The incidence of infection is light in all cases and one would hardly be justified, on the strength of these figures, in saying whether certain varieties are more susceptible to attack than others. Epicure shows the highest percentage infection on a poor yield of small tubers. The two second-earlies, Great Scot and British Queen come next, followed by the other two first-earlies. All the main-crop varieties show a light infection of about the same order throughout, except that Arran Chief is higher than the rest.

The lightness of the attack throughout may have been connected with the good dry summer of 1928 and also with the character and texture of the soil which is a heavy clay loam with many flints. That the character of the soil has an effect on nematodes is proved by the diminution in size of the cysts of *Heterodera schachtii* on potatoes on this heavy land as compared with the normal size of those from potatoes growing in the light loam from Kirton, Lincs., a result which has been demonstrated by Triffitt (1928). Further work, is needed before any conclusions can be drawn as to the susceptibility of different varieties of potato to infection by *T. dipsaci*.

The 20 ft. strip at the east end which was planted with eight rows of King Edward to test for residual infection left in the soil from the 1927 crop gave a yield of 1,552 tubers of which only six were infected with *T. dipsaci* which is equivalent to a 0·386 per cent. infection. This means that for all practical purposes there was no residue of parasites left over in the soil after taking up the 1927 crop. The six diseased tubers may quite well have become infected by the migration of worms into the soil of the area from the two plots twenty-nine and thirty contiguous with it.

(iii.) *Observations on the pathology of T. dipsaci.*

Quanjer (1927) gives the results of extensive observations on the manner in which his polyphagous strain of *T. dipsaci* attacks the potato and other dicotyledonous plants by entering leaves through stomata and stems through lenticels. The worms do not travel inside stolons or up the inside of stems of dicotyledons. He brings out the fact that the conditions of movement within the tissues of a dicotyledon must be different from those in a monocotyledon because of the different arrange-

ment of the vascular system in these two classes of plants. He has also thrown light on the way in which the parasite attacks the tissues of the potato tuber and which particular elements it is that are most affected. It is not the purpose of the present paper to enter into a discussion of his observations but it may be mentioned that one of the principal results he brings out is that the worms cause a dissolution of the middle-lamella substance between cortical or parenchymatous cells whereby the latter easily become separated from one another.

The writer's observations on diseased tubers support Quanjer's findings. It has already been remarked that when a cut is made into diseased cortical tissue the latter is almost mealy in appearance and easily crumbles when touched. This is macroscopic evidence that the cells composing it have become loosened from one another and this fact is further brought out when one takes a small quantity of such mealy substance and drops it on to the surface of water when the cells fly apart from one another either singly or in small groups. This phenomenon has been particularly noticeable when the tissues of certain slightly diseased tubers of King Edward and Arran Chief have been examined in this way. The skin of these had not become cracked or sunken but only looked a little more transparent in one or two patches than over the rest of the surface. When a cut was made under such a patch the tissue was not found to be discoloured but was whiter and more glistening in appearance than the surrounding healthy tissue. A small quantity of this substance which came away from the healthy tissue quite easily, was placed on the surface of water in a shallow glass capsule and immediately the cells separated from one another. When the capsule was placed on the stage of the microscope and examined large numbers of single, somewhat rounded, cells as well as groups of three or four cells were seen lying at the bottom of the water. It was clear therefore that the cement which normally holds healthy cells together had very largely disappeared from between the cells composing this portion of tissue. Amongst such white glistening cells *T. dipsaci* was found in large numbers and in all stages, adults, larvæ and eggs, and they were very active. All the cells seemed to be well filled with starch grains and there was no brown discolouring on the surface of any of them.

In order to test further whether the middle-lamella substance, which is composed of a complex of pectic compounds, had been destroyed or

removed, use was made of an aqueous solution of Ruthenium-red. This, according to Carré and Horne (1927), is specific in its reaction to pectic substances which are stained red by it.

Into a freshly prepared solution of Ruthenium-red, of a deep claret colour, contained in a shallow glass capsule was placed a small quantity of mealy cortical tissue from a lightly affected tuber of King Edward. Many of the cells separated at once and sank to the bottom of the liquid. The material was left in the stain for about 15 minutes after which small quantities of the cells were picked up by means of a pipette and transferred to successive capsules of distilled water so as gradually to get rid of the stain taken up with the cells. Finally some of these were mounted in a drop of clear distilled water and examined under the microscope. Those cells which were completely separated from the rest showed but a very faint pink colouration of the wall; more colour was seen at the points where one or two cells were in contact. For comparison, two thin slices of the healthy part of the tuber were put into Ruthenium-red solution having the same tint and left for 15 minutes, after which they were transferred to clean distilled water and the excess of stain removed. Then two small fragments were broken from the edge of each and mounted in a drop of clear distilled water and examined under the microscope and in these the middle-lamella between adjoining cells was stained a bright red colour. The contrast between the staining reaction of loosened and healthy cells was quite marked and it is evident that the pectic complex of the middle-lamella is profoundly modified by the presence of the parasites; possibly used as food by them.

The worms occupy the intercellular spaces between the loosened cells and the glistening appearance of the affected tissues is probably due to the increased amount of air in it for as the cells become separated from one another the volume of air space is increased. It may be mentioned that the Ruthenium-red test has also been carried out by the writer on the tissues of diseased Narcissus bulbs amongst the cells of which there were large numbers of active *T. dipsaci*. The individual cells were very easily detached from one another and after being in the stain for about half-an-hour the cell walls showed practically no colour at all whereas the cells of healthy tissue were coloured a bright red at the middle-lamella.

The results are interesting as confirming some of Quanjer's observations

and as helping towards an understanding of the problem of the pathogenic action of the worms on plant tissues.

Tylenchus dipsaci ON MANGOLDS.

(i.) *Earlier records.*

Ritzema Bos (1908) gave a detailed account of an attack of *T. dipsaci* on mangolds sent to him for examination from Augustenberg, Germany. The disease only affected the roots towards the end of the growing season when they were almost ready for harvesting. At this time the upper parts of the root began to turn dark in colour accompanied by a rot which gradually affected the whole of the crown so that it easily became detached from the lower part of the root. The disease most frequently started at the level of the soil surface and spread upwards and inwards. The tissues of the root became soft and spongy and assumed a dark brown colour as the disease made headway into them. The epidermis over the affected parts often sank in and became cracked. *T. dipsaci* was found in the discoloured areas along with mites and other secondary invading nematodes, such as *Cephalobus* and *Diplogaster* species in the older rotten areas but *T. dipsaci* alone in the most recently affected parts.

Two pot experiments were set up containing some of the diseased root material; one was sown with rye and the other with onion seed and in both cases some of the resulting seedlings showed disease symptoms and malformations characteristic of the attack of *T. dipsaci*. The worms were also found within the affected parts.

A section of the paper is devoted to a consideration of whether there were earlier records of the parasite attacking mangolds and he concludes that the disease was known to Kühn as far back as 1852. He quotes the account of an obscure disease of mangolds which Kühn gave in his book published in 1858 and shows that the description there given agrees remarkably well with the symptoms and characteristics noted by himself. He also discusses the disease of mangolds described by Vaňha and Stoklasa (1896) and concludes that it was due to *T. dipsaci* though the two Bohemian authors thought they had found some new species of Tylenchus which were the cause. The illustrations which they give of two diseased roots on Pl. 4, figs. 1 and 2, support the view that the disease is the result of attack by *T. dipsaci*.

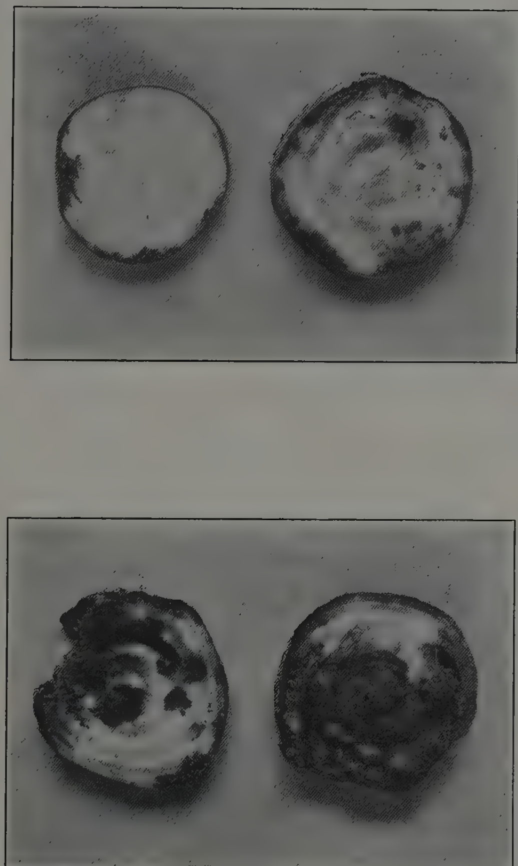
There is one other record of the worms attacking mangolds; Quanjer (1927) p. 160 mentions this crop as being infected on clay soil at Wageningen.

(ii.) *T. dipsaci on Mangolds in Herefordshire.*

About the middle of November, 1927, the writer received two diseased mangold roots which were sent for examination by Mr. Ll. Evans, County Organiser for Agricultural Education, Hereford. They were from a crop grown on a farm close to Hereford where a large number of roots had shown signs of disease. The variety was Red Intermediate and the land on which they were grown was of a light type. By the time the roots reached the writer the crop had been lifted and clamped so that no further information on the appearance of the disease in the field and whether the foliage of affected roots showed signs of disease or was reduced was available. The symptoms of disease on the two roots sent were very similar to those described by Ritzema Bos and one was more severely affected than the other. The skin was sunk and cracked over the diseased parts and in both it looked as though the disease had begun at soil level and had travelled upwards and inwards. On cutting transversely across the root at the site of the disease the tissues were found to be strongly discoloured; instead of being white and red they were of a deep dirty brown tint. In the older affected parts, towards the skin some drying had taken place and here a sort of spongy network of tissue was left. Further in, the tissues were intact but wet and soft and the discolouration had extended over large areas; the appearances being suggestive of an outward spread of discoloured cell-sap from the affected parts into the surrounding substance. Some of the vascular bundles also were discoloured. The accompanying photographs show very well the extent of the damage and the appearance of slices taken across the two roots.

By teasing up some of the affected material in water numbers of *T. dipsaci* were found and when some of it was cut into small pieces and extracted by cold water in a Baermann funnel enormous numbers of the adult worms were obtained.

Some of the material was preserved and some was cut up and put into two pots along with clean loam and sand and seeds of Red Intermediate mangold were sown. The pots were placed in a greenhouse and



Photographs of slices of mangold roots attacked by *Tylenchus dipsaci*; rather less than half natural size.

in due course seedlings appeared but none of them showed any sign of disease either whilst still young or during the whole of 1928 when they grew and produced small roots.

An attempt was made to establish the disease on mangolds on a field plot at Winches Farm on one of the 1/10th acre plots. In March 1928 between 5 and 6 cwts. of diseased roots were obtained from the farmer close to Hereford who had been kind enough to set aside the affected roots as he used the sound ones on opening up the clamp.

On April 19th, the roots were cut up into small pieces by means of a pulping machine and the material thus obtained was distributed as evenly as possible in ten furrows about 6 ins. deep which had been opened up down the length of the plot. The furrows were 2 ft. apart and each was 200 ft. long. The material was covered with soil after it had been put in the furrows. On April 30th seed of Red intermediate mangold was sown the length of each row by means of a "Planet Jr." sower. A good show of seedlings came up and in mid-June the plot was hoed to keep down the weeds. Early in July the plants were singled and more hoeing was carried out. The crop looked very healthy all through the summer and autumn and though the roots were carefully examined every few days no sign of disease appeared on any of them. They were all pulled and topped on October 23rd and during this process each was again carefully scrutinised but no diseased ones were found. It is difficult to suggest any satisfactory explanation to account for the failure to set up the disease both in the pots and on the plot. The pulped material showed the rotted condition plentifully and there can be no doubt that the worms were there. The summer of 1928 was a good dry one in Hertfordshire and this coupled with the transference of the mangold material to the heavy soil at Winches Farm may have proved so unfavourable to the parasites that they could not succeed in bringing about the disease on the crop. This, however, fails to account for the disease not showing on the seedlings grown in pots where one would have expected it to appear. There are, it must be admitted, many as yet obscure points in connection with the setting up of disease in plants by means of infected material.

Host-List of Plants Attacked by *Tylenchus dipsaci*.

Host Plant.	Common Name.	Natural Order.	Authority and Date.
Agropyrum repens	Couch grass	Gramineæ	Ritzema Bos, 1888-92.
Anthoxanthum odoratum	Sweet vernal grass	,,	,, ,,
Avena sativa	Oats	,,	,, ,,
Dactylis glomerata	Cocksfoot	,,	Hodson, 1926.
Holcus lanatus	Yorkshire fog	,,	Ritzema Bos, 1888-92.
Hordeum vulgare	Barley	,,	Darboux & Houard, 1901
Lolium perenne	Perennial rye grass	,,	Rostrup, 1896 (f. Kieffer, 1901).
Poa annua	Annual Poa	,,	Ritzema Bos, 1888-92.
Secale cereale	Rye	,,	Kühn, 1868.
Setaria sp.	Bristle grass	,,	Lagerheim, 1900.
Triticum vulgare	Wheat	,,	Ritzema Bos, 1888-92.
Allium cepa	Onion	Liliaceæ	Beijerinck, 1883.
,, escalonium	Shallot	,,	Darboux & Houard, 1901
,, proliferum	Potato onion	,,	Ritzema Bos, 1888-92.
,, schönoprasum	Chives	,,	,, ,,
,, triquetrum	Triquetrous leek	,,	Gibson, 1929.
,, vineale	Garlic	,,	Ritzema Bos, 1888-92.
Hyacinthus orientalis	Hyacinth	,,	de Vries, 1882.
,, romanus	Roman hyacinth	,,	Prillieux, 1881.
Galtonia candicans	Cape hyacinth	,,	Wakker, 1883.
Scilla campanulata	Squill	,,	Ritzema Bos, 1888-92.
,, cernus	,,	,,	,, ,,
,, sibirica	,,	,,	,, ,,
Tulipa Gesneriana	Tulip	,,	,, 1906
Narcissus tazetta	Bunch-flowered Narcissi	Amaryllidaceæ	,, 1888-92.
,, pseudonarcissus	Daffodils in variety	,,	,, 1917.
Amaryllis formosissima	Jacobean lily	,,	van Slogteren, 1920.
Galanthus nivalis	Snowdrop	,,	,, ,,
Hymenocallis calathina	Sea Daffodil of Peru	,,	,, ,,
Gladiolus hydridus	Gladiolus	Iridaceæ	This record.
Iris xiphium	Spanish Iris	,,	,,
Disa grandiflora		Orchidaceæ	Smith, 1881 (Houard, 1908)
Polygonum fagopyrum	Buckwheat	Polygonaceæ	Kühn, 1867.
,, lapathifolium	Knot-grass	,,	,, 1891.
,, persicaria	Persicaria	,,	,, ,,
,, convolvulus	Black bindweed	,,	Ritzema Bos, 1888-92.
Rheum rhaponticum	Rhubarb	,,	Lebour & Taylor, 1914.
Rumex acetosa	Sorrel dock	,,	Quanjer, 1927.
Humulus lupulus	Hop	Urticaceæ	Percival, 1895.
Beta vulgaris	Mangold	Chenopodiaceæ	Ritzema Bos, 1908.
Chenopodium album	White goosefoot	,,	Quanjer, 1927.
Spinacia oleracea	Spinach	,,	Ritzema Bos, 1917.
Dianthus caryophyllus	Carnation	Caryophyllaceæ	,, 1888-92.
,, plumarius	Garden pink	,,	,, 1904.
Spergula arvensis	Spurrey	,,	,, 1888-92.
Anemone japonica	Japanese windflower	Ranunculaceæ	,, 1903.
,, sps.	Species of bulbous Anemone	,,	Theobald, 1924.
Delphinium ajacis	Larkspur, annual	,,	,, 1913.
Ranunculus acris	Buttercup, Crowfoot	,,	Ritzema Bos, 1888-92.
,, repens	Creeping buttercup	,,	Quanjer, 1927.
Begonia hydrida	Tuberous rooted begonia	Begoniaceæ	This record.
Arabis alpina	Alpine rockcress	Cruciferæ	Quanjer, 1927.
Brassica napus	Rape	,,	Ritzema Bos, 1888-92.
,, nigra	Black mustard	,,	Quanjer, 1927.
,, oleracea capitata	Cabbage, red and white	,,	,, ,,
,, ,, sabauda	Savoy cabbage	,,	,, ,,
,, ,, acephala	Cottager's kale	,,	,, ,,
,, ,, gemmifera	Brussel sprout	,,	,, ,,
,, ,, Botrytis	Cauliflower	,,	,, ,,
,, ,, gongylodes	Kohl-rabi	,,	,, ,,
,, rapa	Turnip	,,	Ritzema Bos, 1888-92.
Camelina sativa	Gold of pleasure	,,	Quanjer, 1927.
Cardamine pratensis	Lady's smock, Cuckooflower	,,	,, ,,
Cheiranthus cheiri	Wallflower	,,	,, ,,
Capsella bursa-pastoris	Shepherd's purse	,,	Ritzema Bos, 1888-92.
Isatis tinctoria	Wood	,,	Quanjer, 1927.
Lepidum sativum	Cress	,,	,, ,,
Raphanus raphanistrum	Wild radish	,,	,, ,,

Host Plant.	Common Name.	Natural Order.	Authority and Date.
Sinapis alba	White mustard ...	Cruciferæ ...	Quanjer, 1927.
,, arvensis	Charlock	,, ...	,, ,,
Stenophragma thalianum ...	Sand rocket	,, ...	,, ,,
Thlaspi arvense	Penny cress	,, ...	,, ,,
Geranium molle	Dove's foot geranium ...	Geraniaceæ ...	Ritzema Bos, 1888-92.
Linum usitatissimum ...	Flax	Linaceæ ...	,, 1903.
Aucuba japonica	Japanese cornel	Cornaceæ ...	Osterwalder, 1901.
Fragaria chiloensis ...	Wild strawberry (Amer.)	Rosaceæ ...	McKay, 1922.
,, elatior	Hautboy	,, ...	Ritzema Bos, 1917.
,, vesca	Wild strawberry (Europe)	,, ...	Quanjer, 1927.
,, indica	Indian strawberry ...	,, ...	,, ,,
Lathyrus odoratus ...	Sweet pea	Leguminoseæ	Theobald, 1912.
Lupinus luteus	Lupin	,, ...	Mayer Gmelin, 1906.
Pisum sativum	Pea	,, ...	Ritzema Bos, 1903.
Vicia faba	Broad bean	,, ...	,, 1888-92.
,, sativa	Tares, common vetch ...	,, ...	,, 1906.
Phaseolus vulgaris ...	Kidney bean	,, ...	,, 1912.
Trifolium pratense ...	Red clover	,, ...	Kühn, 1881.
,, repens	White clover	,, ...	Ritzema Bos, 1912.
,, incarnatum ...	Crimson clover	,, ...	Darboux&Houard, 1901.
Medicago sativa	Lucerne, alfalfa	,, ...	Kühn, 1881.
Anthyllis vulneraria ...	Kidney vetch	,, ...	Amos, 1919.
Onobrychis sativa	Sainfoin	,, ...	,, ,,
Primula sinensis		Primulaceæ ...	Ritzema Bos, 1903.
Lysimachia sp.		,, ...	,, 1913.
Anagallis arvensis	Scarlet pimpernel ...	,, ...	Hall, 1902.
Convolvulus arvensis ...	Lesser bindweed ...	Convolvulaceæ	Quanjer, 1927.
Phlox decussata	Herbaceous phlox ...	Polemoniaceæ...	Ritzema Bos, 1899.
,, drummondii ...	Annual phlox	,, ...	,, 1904a.
Calceolaria rugosa	Calceolaria	Scrophulariaceæ	This record.
Veronica serpyllifolia ...	Thyme-leaved speedwell	,, ...	Quanjer, 1927.
Chelone glabra		,, ...	Osterwalder, 1902.
Myosotis stricta	Forget-me-not	Boraginaceæ ...	Ritzema Bos, 1888-92.
Solanum tuberosum ...	Potato	Solanaceæ ...	Kühn, 1888.
Nicotiana tabacum ...	Tobacco	,, ...	Schœvers, 1917.
*Plantago lanceolata ...	Ribwort plantain ...	Plantagineæ ...	Ritzema Bos, 1888-92.
Dipsacus fullonum ...	Teasel, fuller's thistle ...	Dipsaceæ ...	Kühn, 1858.
,, sylvestris ...	Wild teasel	,, ...	Havenstein, 1880.
Saxifraga cotyledon ...		Saxifragaceæ ...	This record.
Bellis perennis	Common daisy	Compositæ ...	Ritzema Bos, 1888-92.
Crepis taraxacifolia ...	Beaked crepis	,, ...	Maige, 1906.
,, virens	Smooth crepis	,, ...	Godfrey, 1924.
,, fœtida	Fœtid crepis	,, ...	Houard, 1912.
Centaurea cyanus	Cornflower	,, ...	Kamrodt, 1867.
,, jacea		,, ...	Ritzema Bos, 1888-92.
Cynara cardunculus ...	Cardoon	,, ...	Stefani Perez, 1912.
Cirsium arvense	Creeping thistle ...	,, ...	Quanjer, 1927.
,, lanceolatum ...	Spear thistle	,, ...	,, ,,
Senecio vulgaris	Groundsel	,, ...	,, ,,
Sonchus oleraceus ...	Common sow thistle ...	,, ...	Ritzema Bos, 1888-92.
,, asper	Shaggy sow thistle ...	,, ...	Quanjer, 1927.
Hypochœris radicata ...	Cat's ear	,, ...	Godfrey, 1924.
Taraxacum officinale ...	Dandelion	,, ...	,, ,,
Hypnum cupressiforme ...	Moss	,, ...	Ritzema Bos, 1888-92.
Syringa vulgaris	Lilac	Oleaceæ ...	Steiner, 1927.
*Scilla nutans	Bluebell	Liliaceae ...	Ramsbottom, 1918.
*Plantago maritima ...	Seaside Plaintain ...	Plantagineæ ...	Hodson, 1929.
*Plantago major	Greater Plaintain ...	,, ...	,, 1929.

The foregoing list of plants on which *T. dipsaci* has been found causing small galls or other localised swellings on leaves and stem or more extensive damage accompanied by rot, has been compiled from the earlier lists of Marcinowski (1909) and Ritzema Bos (1917 and 1922) with additions from Quanjer (1927) and other records which have come to the writer's notice.

It is quite possible that further additions to it will have to be made when we know definitely whether it is *T. dipsaci* which is responsible for the galls on several wild plants listed by writers on plant galls. There are numerous records of such galls in which Tylenchus sps. occur. Marcinowski (1909) pp. 176 and 177 gives a good number and the great work by Houard (1903-1913) contains a large number of them. Godfrey (1924), p. 474, gives a list of wild European Composites showing nematode galls compiled from various sources and concludes that there is good reason to consider all of them to be due to *T. dipsaci*. He has in fact found galls on the true dandelion *Taraxacum officinale*, caused by *T. dipsaci*. They were found on this host many years before by Thomas in 1885 and the worms cited as Tylenchus sp.

A few words also are necessary about certain of the plants listed above.

Corms of Gladiolus sp. and bulbs of Spanish Iris are recorded as hosts for the first time. In the former the worms were found in 1928 at the Ministry of Agriculture and Fisheries Plant Pathological Laboratory, Harpenden, by Mr. Buckhurst and Mr. Moore who informed the writer. The parasites were found in Spanish Iris bulbs by the writer in November, 1924, in diseased material sent in for examination. In the case of Begonia, which is a new record for the parasite, Mr. Buckhurst found the worms in diseased tubers in 1928.

The case of *Allium triquetrum* is interesting as the plants were growing in a bed where Narcissus was being killed out by the parasite in the Isles of Scilly. The record on Rhubarb calls for some comment. This plant is largely grown on a commercial scale round Leeds where it is subject to a serious disease known as "Crown Rot." In the decaying, discoloured tissue of the root stock *T. dipsaci* occurs plentifully. Its presence there was recorded by Lebour and Taylor (1914) in a letter to "Nature." Material was sent to the writer by Mr. Taylor in 1920 and again in 1929 and on both occasions *T. dipsaci* has been found. "Crown Rot" in rhubarb has, however, been the subject of an important piece of bacteriological research as a result of which Millard (1924) shows that the disease is caused by a micro-organism, *Bacterium rhaponticum*. It is possible, however, that *T. dipsaci* helps in the spread of the disease-producing bacteria as both Taylor and the writer have found the worms at the advancing edge of the disease.

In the case of the common *Calceolaria* the writer found *T. dipsaci* in the discoloured leaves of a plant sent to the Institute for examination in May, 1928, by Mr. H. W. Miles of Manchester University.

On *Saxifraga cotyledon* the parasites were associated with a rot of the crown and a blackening of the leaves in the case of some plants sent to the writer by Mr. Fox Wilson from the Royal Horticultural Society's Gardens at Wisley in November, 1928. The worms recorded on lilac by Steiner were found on the roots; a most unusual situation for this parasite.

The writer desires to express his thanks to Dr. Pethybridge of the Ministry of Agriculture Plant Pathological Laboratory, Harpenden, for the gift of a copy of his translation of Dr. Quanjer's paper, and to Messrs. Buckhurst and Moore of the same Laboratory for help with some of the records in obscure journals.

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A New Type of Wedge Colorimeter used in a Biological Investigation of Sewage.

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INTRODUCTION.

FOR two years, from June, 1927, the writer held a Research Scholarship in Sanitary Science from the Grocers' Company, the terms of reference including a biological study of the fauna of sewage-treatment plants. Special attention was paid to the free-living nematodes since in mere quantity they form an important section of the fauna in many treatment units, and they have been much neglected as a group in comparison with the protozoa and bacteria.

In the course of the investigations it was found desirable to measure various physico-chemical factors, such as the hydrogen ion concentration, oxygen absorption and turbidity of sewage liquors, to see if correlations were revealed between any of these factors and the distribution of organisms in the sewage. Simple tests were employed at first but, as these proved insufficiently delicate, a more accurate technique was elaborated. Particularly in the matter of hydrogen ion determinations, by colorimetric methods, the need for a useful colorimeter was felt, and was partly met by the apparatus described in the present article. Subsequently the colorimeter was adapted to the measurement of oxygen absorption and turbidity.

The investigation was not primarily concerned with physico-chemical factors, and a technique was therefore required which should be at once simple and rapid in application while yielding results of sufficient accuracy for the purposes in hand. It is suggested that the colorimeter described constitutes a slight approach towards such an ideal technique.

The principles of the device and the growth of the idea are briefly characterized first, the actual construction of the apparatus is then given in detail and finally its use and application to specific samples are illustrated.

The writer is deeply indebted to the Grocers' Company for making the investigation possible and for liberally supplying glassware. He would particularly wish to thank the Assessor, Sir John Rose Bradford, F.R.S., and Professor R. T. Leiper, F.R.S. under whose supervision the Scholarship was held, for their interest and helpful advice.

Principles.

Most of the indicators commonly used for the colorimetric determination of hydrogen ion concentrations—and especially those indicators of the sulphone-phthalein group—present a simple colour change from one definite acid colour, through intermediate tints, to another definite alkaline colour. For example, bromo-thymol blue changes from yellow through green to blue, and phenol red from yellow through orange to red. If a certain depth of liquid containing the yellow form of phenol red be superimposed upon a depth containing the red form, in a system of glass tubes, then the resulting orange virage will be found to match that seen through a solution of intermediate pH-value and colour. If equal concentrations of indicator are used in the yellow, red and orange solutions and the conditions are such that Beer's law is obeyed, then the depth of the orange solution will be equal to the sum of the depths of the red and yellow solutions. Thus if this latter sum be kept constant and its components allowed to vary, it will be possible to match any intermediate shade from red to yellow. This principle has been employed by Gillespie (1921), with a complication of whose colorimeter the writer has had some experience on a previous occasion (see fig. 1). In the figure, A to E are glass tubes with optically plane glass bottoms. In Gillespie's colorimeter A, C, D and E are fixed, A and D being a constant height (*a*) above C and E, while B is movable vertically and actuates

a pointer moving over a scale. The solution of intermediate tint (and unknown pH-value) is placed in E, and the extreme colour-forms in B and C, the three liquid surfaces being broken in each case by the tube next above so that no meniscus effects are possible. The two virages are now viewed vertically downwards (by a system of mirrors they can be placed in juxtaposition for easier comparison, and by another mirror light can be reflected upwards through the tubes), and the tube B is adjusted until an exact colour match is obtained. In the colorimeter formerly used by the writer (ingeniously invented and constructed,

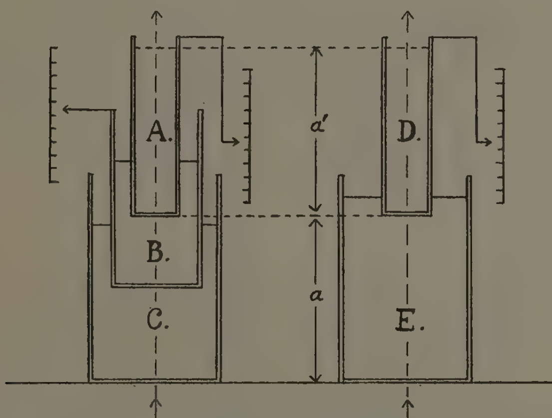

Fig. 1.—Diagrammatic elevation of a colorimeter of the Gillespie type.

with dissecting-microscope stands carrying the tubes in the lens-holders, by Mr. W. Wakefield of Bristol University), the tubes A and D were also movable. For it was found that the depth in E was not always exactly equal to the sum of the depths in B and C, with some indicators, and experiments were made to investigate this discrepancy; moreover, by this means different readings could be taken at different total depths.

Good results can be obtained with a colorimeter of this type when transparent and colourless liquids are to be tested with bright indicators possessing a considerable change in colour quality. But with solutions more or less turbid or initially coloured it becomes increasingly difficult

to obtain a good colour match. A way out of the difficulty is provided by the comparator device due to Walpole, and already described by the writer in this Journal (1926, p. 94). As applied to Gillespie's colorimeter, the device works as follows. The turbid (or coloured) solution, with added indicator is placed in E at a fixed depth (a). Some of the same solution but without indicator is now added to the viewing-tube A, and plain water to D, to an equivalent depth (a^1). The sums of the total constituents on both sides of the apparatus are now the same, and accurate colour matching is possible if the resulting virages are not too obscure. But now at the top of A and D is produced a meniscus effect which can be overcome only by adding another pair of viewing-tubes. In Wakefield's variation of the colorimeter, with A and D movable, the additional tubes would also have to be movable, producing an apparatus of quite unwieldy complexity. In the present investigation dealing with sewage liquors some degree of turbidity and colour were the rule rather than the exception, and an attempt was therefore made to construct an apparatus which should combine the principles of the Gillespie colorimeter and the Walpole comparator without undue complication.

As a result of the necessity described the writer stumbled upon the idea of a pair of fixed glass wedges instead of the tubes A, B and C of the Gillespie colorimeter, the wedges to be arranged in the form of a rectangle (see fig. 2), and the virage to be observed laterally through slits (in the contrary direction of the arrow). Then, assuming phenol red to be the indicator with the red form in one wedge (B) and the yellow in the other (C), by moving the slits to the extreme left a red virage would result—to the extreme right a yellow virage, and in intermediate positions all the intermediate shades of orange. A rectangular glass box, of the same fluid width as the wedge-couplet, would provide the second system to contain the liquid under test (E). Two more similar glass boxes are required, one to be placed in the line of sight of the first system to contain turbid solutions (A) and one in the second system to contain a water blank (D), and the analogy with the Gillespie colorimeter is complete (see fig. 1). There remain only the problem of producing and measuring a movement of the slits or of the wedges, and that of rendering the virages in the two systems readily comparable.

The apparatus had already been constructed when the writer happened

upon the descriptions of two previous wedge colorimeters, namely by Barnett & Barnett (1921) and by Myers (1922) respectively.

Myers appears to hang a series of *vertical* wedges one behind the other, using one for ordinary colorimetric work, two for bicolorimetric work, and a third for turbid solutions (presumably as a comparator). He says: "The reading of the wedge containing the dominant colour of the dye, *e.g.*, the red in phenol red, characterizes the hydrogen ion concentration, the yellow wedge being employed simply to obtain a correct colour-match. This being the case, it may also be employed to correct for any slight error due to extraneous yellow pigment in the unknown." He would seem to ignore the dispersive effect of the wedges, an effect which would be considerable when all three were in use, and his comparator device appears not to possess the nice balance of constituents to be found in that of Walpole.

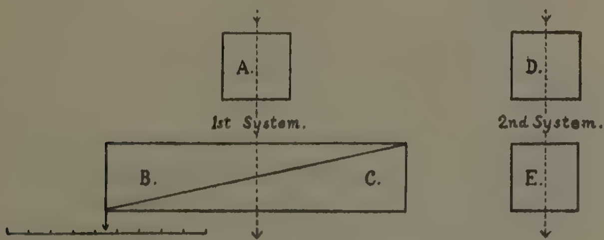

Fig. 2.—Diagrammatic plan of the wedge colorimeter. Compare fig. 1: in the two figures similar letters stand for functionally similar units.

Barnett & Barnett use a rectangular glass box, of dimensions 35 cm. long, $1 \cdot 5$ cm. fluid width and $2 \cdot 5$ cm. high, with a diagonal glass partition. A scale is fixed along the lower edge of the nearer wedge and is graduated directly in pH-differences of $0 \cdot 1$. Readings are then added algebraically to the mid-point pH-value of the indicator in use. No mention is made of viewing slits in their article, and there is no clear description of the size and position of the test-vessel; presumably it is placed on top of the wedges and slid along until its virage can be matched with a section of the graduated virage produced by the wedges, no slits being used. The writer has found a mask with slits very helpful, since it cuts out all

but one narrow band of the graduated virage. However, in the Barnetts' colorimeter the wedges are of such a length (35 cm.) that a colour change corresponding to a pH-difference of 0·1 would extend about 2 cm. when working towards the middle of the range of the indicator (towards the extremes of the effective range the value would fall to about 0·7 cm.), so that matching would not be unduly difficult even without slits. A comparator device is also mentioned by them: "Compensation for coloured or turbid solutions is made by placing a small glass compartment of the same fluid diameter behind that portion of the comparator [*i.e.*, the wedges] in which the match is to be obtained." Altogether their colorimeter is practically identical in principle with that independently devised by the writer; but it may be of some use to give constructional details of the latter since these are very meagre in the article describing the former.

Construction.

The colorimeter consists of a framework built up of "Meccano" parts, screwed down to a wooden base board and coverable by a box of stout millboard. The framework is 24½ inches long, 5½ inches wide and 9½ inches high, and, being composed of "angle girders," is quite rigid. The glass vessels are made of slabs of optically plane glass cemented together with a substance that is acid-proof and chemically inert. They are: three boxes of 3·0 cm. square cross-section and 6·0 cm. high; and two wedges 16·0 cm. long, 3·5 cm. wide at the base and 6·0 cm. high (the dimensions are internal, the glass being 0·2 cm. thick).

At about half the length of the framework are two transverse guides on the floor, between which are placed a box containing the solution under test, and a second box containing water if the comparator is in use (these represent the second system, D and E, in fig. 2). At about half the height of the framework is a skeleton platform to support the wedges and the comparator vessel (the first system in fig. 2) immediately above the vessels of the second system. The comparator vessel is held in transverse guides, distal to the observer. The wedges are mounted on a movable trolley, 9½ × 2½ inches, at the corners of which are four

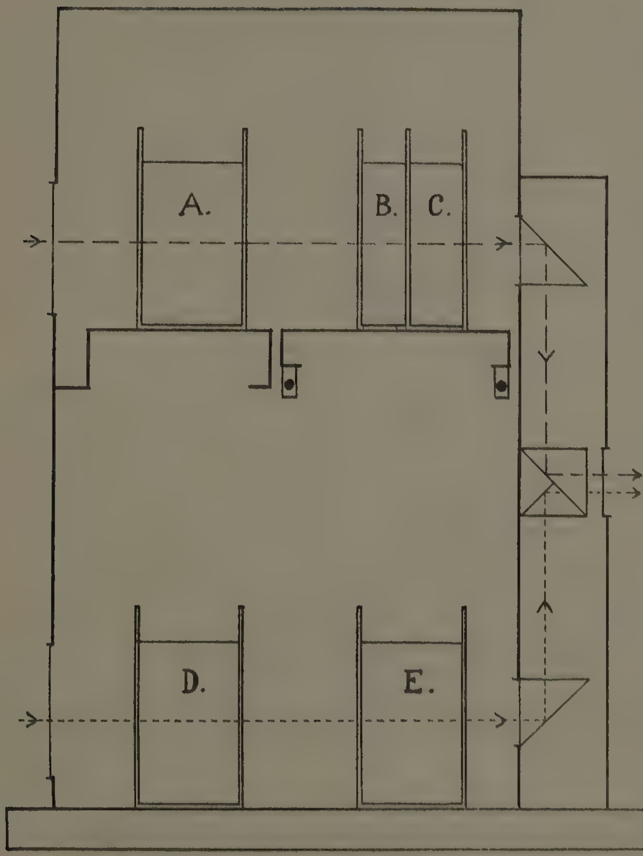

Fig. 3.—Median transverse section of the wedge colorimeter; compare plan in fig. 2.

collars sliding along two steel rods in the direction of the length of the apparatus. The trolley is actuated on the endless chain principle, a sprocket chain passing from one side of it over toothed wheels at each end of the framework and back to the other side. The toothed wheel on the observer's right is small ($\frac{3}{4}$ inch) and is actuated by hand by turning a milled wheel projecting outside the framework. The toothed wheel on the left is large (3 inches) and actuates a long pointer (made by drawing out a glass rod and blackening the point with Chinese ink) which moves over a circular scale outside the framework. The small actuating wheel admits of fine adjustment: the large registering wheel ensures that the pointer, in moving between its limits, covers not more than one complete rotation. This sprocket chain system has proved remarkably free from backlash, the pointer being responsive to the slightest movement of the milled wheel. This completes the mechanical part of the apparatus.

On the sides of the framework, opposite the fixed boxes of both the first and second systems, are two masks in which vertical slits are cut allowing light to pass through the systems. In each mask the upper slit is nearly, but not quite, vertically above the lower. Outside the mask on the side nearer the observer is fixed a plate of glass bearing four right-angled prisms. One of these is cemented over the upper slit so as to reflect the light vertically downwards, another over the lower to reflect the light upwards. Midway between them the other two are cemented side by side, so as to reflect the light outwards (see fig. 3). The prisms are covered by a box having a single central window through which the images of the slits are observed. Since the slits in each pair are slightly out of vertical alignment it is possible to bring the images of the upper and lower slits into juxtaposition, and colour matching is greatly facilitated. Over the slits on the far side of the framework is fixed a sheet of thin translucent paper, which may be coated with phenol red to act as a colour filter when using artificial light, as described by Clark (1922, p. 67). When the apparatus is in use a millboard box is dropped over it to cut out extraneous light.

The width of the slits is a feature of some importance considering the graduated virage presented by the wedges. The effective length of the latter, 13·5 cm., corresponds to a pH range of about 2 units for most indicators, and, as the pH-change per unit length becomes more

rapid at the extremes where the colour change is slightest, the effective pH-range is reduced to about 1·5 units. Each of the nearer slits is 0·3 cm. wide and 2·0 cm. long. As the optical distance from the eye to these is 13 cm., and from the eye to the further slits 27 cm., the latter have to be of about twice the dimensions (hence the advantage of keeping the wedges on the near side). It has been found that the width of the slit covering the wedges corresponds to a pH-difference of about 0·04 units towards the centre of the effective range of the indicator in use. In this region the colour change is most marked, but the writer at least is unable to appreciate a colour change corresponding to less than 0·05 pH-units under optimum conditions, so that the slit is sufficiently narrow to present a virage which appears quite homogeneous. For much wider slits it would be necessary to use proportionately longer wedges.

Mention has already been made of the fact that, in working with a colorimeter of the Gillespie type, the total fluid depths on each side of the apparatus were not always exactly equal at the matching point. It was for this reason that two separate glass wedges were obtained for the present colorimeter, since by sliding them one against the other the fluid depth could be made greater or less than that of the test vessel. Experience showed that for practical purposes this adjustment was both awkward and unnecessary, useful as it might be in investigating the theory of indicators. Accordingly, the two wedges were sunk into a template of stout millboard in such a position that their combined fluid depth was exactly equal to that of the test vessel (3·0 cm.). In this position the effective length of the wedge couplet was reduced, by overlap, to 13·5 cm., corresponding to a rotation of the pointer through 194 degrees.

After the calibration of the apparatus interchangeable dials were made for use with different indicators and for other purposes, so that the pH-values and other readings could be taken directly. When a dial was changed no adjustment of the pointer was usually necessary, but this could nevertheless be simply effected by moving the wedges along to one extreme position, loosening the pointer and setting it at zero on the dial by means of a set-screw.

Calibration.

The indicator solutions in the wedges should have hydrogen ion concentrations respectively above and below the pH-limits of the indicator. Granted this condition, the precise pH-value of these solutions matters little. This is one of the advantages of this type of colorimeter; slight disturbing influences such as alkali from the glass, which might seriously affect a test made with ordinary buffer tubes, need not be considered, and the solutions can be kept without deterioration for some days.

Nevertheless, the writer has found it desirable to use more or less definite standards. At high acidities, for instance, both phenol red and neutral red undergo secondary colour changes. The limits between which it is practicable to use a given indicator are recorded in text books or on the bottle. The actual limits of colour change extend further than this, however, so that such standards must be chosen as will allow substantial margins beyond the effective range. For a purely subjective reason, the two margins are not equal in extent. The eye can more easily detect small quantities of a strong colour (red or blue) in a weak colour (yellow) than *vice versa*, so that a wider margin should be allowed on the side of the strong colour (the alkaline side, for most of the sulphone-phthalein indicators). Thus, working with bromothymol blue which has an effective range of pH 6·0 to 7·6, the writer used standard solutions in the wedges of pH 5·4 and 9·0. As a matter of fact, it would be possible to use the effective limits as standards, provided those standards were rigidly maintained; the whole point of shifting the standards away from the effective limits is that slight changes in their reaction will not perceptibly affect the results. For preparing the standards the British Drug Houses' "Universal" buffer solution was used.

It is now necessary to prepare solutions buffered at selected points throughout the effective range of the indicator to be calibrated. Intervals of 0·2 pH-units are satisfactory for the middle of the range; in this region the graphical plot of percentage colour against pH-value is a fairly straight line. Towards the extremes of the range, where the curve flattens out, intervals of 0·1 pH-units are desirable. It is worth while using a more restricted and accurate buffer mixture for this purpose,

e.g., Clark and Lubs' " Phosphate—NaOH " solutions for bromo-thymol blue. These standards are successively placed in the square box on the ground floor of the apparatus and the wedges adjusted until an exact match obtains, the pointer readings (preferably in degrees of rotation) providing the material for constructing a dial calibrated directly in pH-units. This procedure must of course be repeated for each indicator to be used. In the sewage investigations phenol red and bromo-thymol blue were sufficient for the measurement of all the samples collected.

Operation.

A quantity of the standard solutions sufficient to cover the slits is added to each of the wedges, 100 c.c. each in the present case. Similarly, to sufficient of the sample under test (30 c.c.) is added the requisite amount of indicator. This amount should be as small as is consistent with obtaining a clear colour judgment ; for many sewage liquors it was found necessary to use 1 drop of a 0·04 per cent. solution for each cubic centimetre of the sample, and this concentration was therefore taken as standard for the wedges and all samples.

For all but very transparent samples it will be necessary to use the comparator device. A further 30 c.c. of the sample, but without added indicator, is placed in a box behind the wedges, and a water blank is placed behind the sample being tested.

Some samples are so turbid that colour matching is impossible. If electrometric methods of measurement are not available, moderate dilution of the sample can be resorted to.

It is important that all tests should be made at about the same temperature, namely the temperature at which the apparatus was calibrated for a particular indicator. The milled wheel must be turned with a slow steady movement, or waves will be set up in the wedges causing the standards to overflow and mix.

Adaptation for Measuring Turbidity.

Techniques have been recorded for artificially producing turbid solutions, the absolute turbidity of a given sample being expressed in

terms of the artificial standard. No such measurements were made by the writer, though the colorimeter readily lends itself to the purpose. However, a relative measurement was occasionally made to ascertain the reduction in turbidity of a sample after centrifuging or settling. For this purpose 100 c.c. of the untreated sample are run into one of the wedges and water into the other. The treated sample is placed in the lower box and a direct reading taken on a dial graduated in 100 parts; the reading gives the percentage reduction in turbidity provided that the water and untreated sample have been run into the appropriate wedges. The untreated sample in the wedge should be stirred repeatedly.

Adaptation for Measuring Oxygen Absorption.

It was desirable to make rough and rapid estimations of the oxygen absorption shown by various sewage liquors so as to obtain relative values of their putrescibility, with a view to ecological classification of the contained organisms. Purvis & Hodgson (1922, p. 55) give details of a standard estimation for oxygen absorbed in three minutes at 80° F. using an acid solution of potassium permanganate and titrating with oxalic acid. Kershaw (1925, p. 214) gives a rough test based on the foregoing, in which to 25 c.c. of sewage effluent are added 10 c.c. of 10 per cent. sulphuric acid and 1 c.c. of N/80 permanganate; if the mixture is not decolourized in 3 minutes the effluent may be regarded as good. If 2 c.c. of permanganate are decolourized in 3 minutes the effluent is poor or bad.

The standard estimation was too lengthy for the purpose in hand. Kershaw's test proved useful as a rough guide, and was easily carried out at the sewage works, but it was insufficiently delicate for allotting relative values to the various samples. The colorimeter, however, was easily adapted so as to make Kershaw's test quantitative. The principle of his test is to measure the time interval at the end of which all trace of colour has disappeared (an end-point difficult to fix with any accuracy); the principle of the colorimetric method here described is to measure the degree of colour remaining after fixed intervals of time have elapsed.

The quantities used are: 2 c.c. N/80 $KMnO_4$ per 25 c.c. liquid (water, in the case of the wedge: sewage, in the test vessel) with the addition

of 10 c.c. of 10 per cent. H_2SO_4. This is equivalent to 1 c.c. of Normal $KMnO_4$ per litre, or 8·0 mgm. oxygen per litre (0·562 grains per gallon).

Calibration was effected by pouring the above solution (in distilled water) into one of the wedges with water in the other, and adding successive dilutions of it to the test vessel. This revealed a practically straight-line relationship between rotation of the pointer and percentage colour, so that a dial was simply constructed reading directly in milligrammes of oxygen absorbed per litre (from 0 to 8).

The procedure is to prepare the standard for the wedge, mix the solution to be tested noting the exact time at which the $KMnO_4$ was added, stir thoroughly and place the test vessel in the colorimeter. By taking readings at a series of definite times it is possible to plot oxygen absorption against time graphically, the resulting curve being a more interesting statement of the reaction than a solitary value. As a routine procedure the test was stopped at the end of 10 minutes, though with some samples it was prolonged over one or two hours. It is important to keep the temperature of the room constant throughout the test, and for all tests the results of which are to be compared. Failing this it would be necessary to apply a temperature correction factor. The comparator device is, of course, applicable in the case of turbid samples.

For some crude sewage samples it was found that the 8 mgm. of oxygen per litre were absorbed completely before there was time for a reading to be taken. In this case double or treble quantities of $KMnO_4$, and proportionately stronger acid, were added, the pointer readings being multiplied accordingly.

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On the Occurrence and Significance of *Heterodera schachtii* infesting certain Weeds.

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Since the discovery of the plant-infesting nematode *Heterodera schachtii* Schmidt, 1871, many infections of this nematode occurring in different countries on a wide range of host-plants have been studied. Owing to the serious agricultural losses occasioned by this pest, efforts have been made, both in Europe and in the United States, to determine the range of plants which are susceptible to infection. Lists of plants susceptible and non-susceptible to attack have been drawn up by various workers, and these have comprised not only plants of economic importance, but also weeds which might serve as an important means of propagating the parasite. The abundance of contradictory evidence which appears in these lists is only explicable by Steiner's (1925) exposition of the hypothesis of biological or physiological strains. This work serves to emphasise the importance of crop rotation as a means of combating the parasite, more particularly when the nematode population constitutes a monophagous strain, *i.e.*, one which has become highly specialised upon a single host-plant and has lost the power of readily attacking other species. Where a polyphagous strain occurs, the range of non-susceptible plants which can be used in the course of the rotation is greatly diminished, and, in such cases, the weeds occurring on the infected land may add an important complicating factor to the problem of eradicating the pest.

Recently Goffart (1928) has shown that, in addition to physiological specialisation, the parasite may show some morphological distinctions which are constant for all individuals developed on a single species of

host. Thus, in all the infections upon the potato-plant which have so far been described, the mature females, or cysts, have been rounded in contour, while on other hosts the cysts are more elongated in shape, with a caudal projection bearing the vulva. That this is merely a host reaction has been proved by experiment.

Field Observations on *H. schachtii*.

Three infections of *H. schachtii* in Britain have been studied under field conditions by the present writer. Of these, two formed natural infections on potatoes, the third attacked mangolds and cauliflowers. The strains attacking potatoes occurred in Lincolnshire and Lancashire and both appeared to be very highly specialised on this host.

The Lincolnshire strain was transplanted to a plot of land in Hertfordshire on which a large variety of weeds commonly occurred. Potatoes were grown for three successive years on this plot, and such weeds as were present were examined, but proved to be free from infection. After this period when the infection was well established, a portion of this plot was allowed to lie fallow and unweeded and again careful examinations of the weeds were carried out. In no case was any species of weed found to be infected, and it was assumed that, despite the absence of the natural host, the nematode was unable to establish an infection upon any of the numerous plant species present. Pot experiments were set up in an attempt to produce infection with this strain on various cultivated plants. Sugar-beet seedlings were grown repeatedly in infected soil for a period of three years, during which time the roots were frequently examined but with negative results. Several species of the order *Solanaceæ* were grown in Lincolnshire soil with a heavy cyst content, and these also gave negative results. The following species were thus tested :—

Solanum crispum.	*Hyoscyamus niger.*
Solanum capsicastrum.	*Nicotiana tabacum virginica.*
Atropa belladonna.	*Petunia hybrida alba.*
Salpiglossis grandiflora.	*Browallia elata nana.*
Physalis alkekengi.	*Egg Plant.*

Morgan (1925) found a single plant of *Chenopodium album* growing under natural conditions in a potato field in Lincolnshire to be slightly infected with the nematode, and he was also successful in establishing a slight infection on tomato.

The Lancashire strain, which has more recently come under observation, occurred on the Ormskirk Potato Testing Station, a branch of the National Institute of Agricultural Botany. It was found to form a very heavy infection on land which had been under a rotation of crops for eight years. The infection on this land had not previously been observed, and other crops had been satisfactory. The sudden and intense infection, accompanied by failure of the potato crop was, therefore, somewhat unusual, more especially as it occurred over a large area and did not display the patchiness common in fields where an infection of *H. schachtii* is gradually becoming established. The cyst content of the soil was found to be heavy throughout the affected area.

Morgan (1929) has recorded that, in the case of a Lincolnshire field where the failure of the potato crop had been associated with a heavy infection of *H. schachtii*, a rotation period of four years, during which non-susceptible crops were grown, was sufficient to produce a satisfactory yield when, in the fifth year, the land was again cropped with potatoes. He has further pointed out that the eel-worm had remained viable during the period of rotation, and formed a considerable infestation on the roots of the potatoes. Since the Ormskirk field had been under rotation for double this length of time, it seems safe to assume that the failure of the crop was not solely due to an infection which might have become established on the previous potato-crop and remained dormant in the soil for the eight intervening years.

A survey of another field belonging to the Station, from which the eighth crop in the rotation (a cereal), had recently been harvested, and which was to be planted with potatoes the following year (1929), showed that a very slight infection was present throughout. The cysts in this field, proved, however, to be mainly ovoid in shape, thus differing from the type produced by nematodes parasitic upon the potato. This suggested that some other plant-species might be involved as a host, and consequently as a means of propagation of the parasite. Two possibilities therefore arose, either that one of the crops used in the rotation was susceptible, or, that one or more species of weed which occurred naturally on the land was attacked by the nematode, and served not only to maintain but to increase the infection during the rotation period. Of these two suggestions the second seemed the more probable since the nematode would have more opportunity of becoming adapted to a weed which

occurred constantly, than to an agricultural crop which was not grown annually.

One portion of this lightly infected field is, this season, under potatoes and the remainder under oats, the two crops being separated by a strip of uncultivated land wide enough to form a cart-track. This strip has been found to harbour a considerable variety of weeds, and the roots of these have been carefully examined for cysts. Of these weeds, one species only, *Agropyrum repens* (Couch grass), has been found to be infected, but on this the infection was fairly constant and moderately heavy. Furthermore these cysts, on microscopical examination proved to be ovoid and to resemble in shape and size the brown cysts previously extracted from this soil. This observation leaves little doubt that, in this instance at least, the infection was maintained and increased by the weed, and that this strain, although highly specialised on potato had not completely lost the power of attacking other plant-species.

It is of some interest, and possibly of some significance, that a polyphagous strain attacking mangolds, included *Agropyrum repens* and two other varieties of grasses amongst the list of weeds on which it was found to occur under field conditions. It has also previously been recorded as a host of *H. schachtii* by Tarnani (1898). It should be noted that Couch grass was not present on the Hertfordshire plot, so that its susceptibility to the Lincolnshire strain has not been tested. A very small percentage of ovoid cysts have been detected in Lincolnshire soil, the presence of which may be due to infections on *Chenopodium album*, or some other weed at present unidentified.

A Heterodera Species forming a Natural Infection on *Psamma arenaria*.

This infection was discovered by Mr. W. E. H. Hodson, to whom I am indebted for the material from which these observations have been made. Cysts were discovered by him to be plentiful on the roots of *P. arenaria* (sea maram grass) on a sand-dune at Dawlish Warren. Since the original material was obtained, this dune has been destroyed by a gale, but lighter infections have been found on the same host growing in more sheltered positions. As the material originally collected had been preserved, fresh roots bearing cysts were kindly supplied by Mr. Hodson. These were planted in sand, but unfortunately the infection, originally slight, died out. The following description has therefore been compiled from preserved material only.

The cysts, which occurred chiefly on the lateral rootlets, were indicated only by small aggregations of sand-grains adhering firmly together. On microscopical examination the sand-grains could be seen to be partially embedded in a colourless, gelatinous substance. After removal of the sand this substance was found to be of a firm consistency and

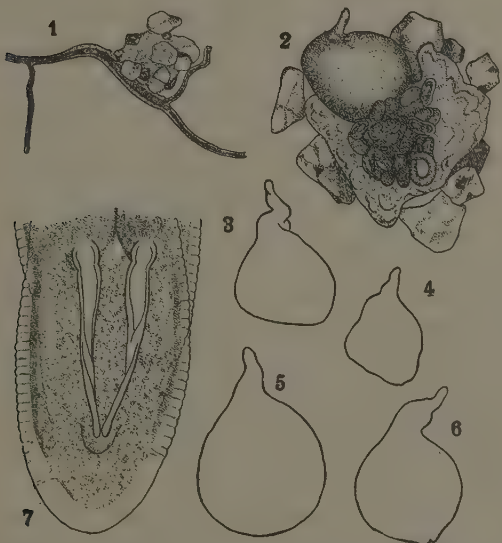

Fig. 1.—Root of maram grass bearing sand-covered cyst. (X 20.)
Fig. 2.—Dissection showing cyst, eggs, and adult male. (X 50.)
Figs. 3-6.—Isolated cysts. (X 50.) Fig. 7.—Tail of adult male. (X 1000.)

to be laid down in faintly defined, concentric layers. A further dissection revealed a mass of eggs and an adult male nematode, and finally the female worm, or cyst, attached to the root. Frequently more than one cyst was found embedded in the mass, as many as five being grouped together in some instances. In these cases the number of males was always found to correspond with the number of females.

Where single cysts were present, counts of the extruded eggs were

made, and these were found to vary greatly in number from less than a dozen up to forty-eight. The quantity of gelatinous material and the stage of development of the eggs also varied for different individuals. Where many eggs were present the gelatinous substance was abundant and a large proportion of the eggs were embryonated. Dissection of the cysts showed that the genital organs were in almost every case distinguishable and the internal cavity was not completely filled with eggs. From this it was assumed that the cysts had been preserved before reaching maturity. All the eggs within and around one solitary large-sized individual were found to number 290.

The presence of the adult males within the egg-mass suggests that fertilisation may take place outside the body, or that it occurs at intervals of time, or that immediately following copulation a mucilaginous mass is ejected by the female in which the male becomes entangled and into which the eggs are later extruded. As against this third possibility is the fact that in every case the males were found to be in good condition and showed no signs of decomposition.

Measurements were taken of the various stages of the life-cycle of this nematode to discover whether any striking differences occurred between it and the various strains of *H. schachtii* which have been described. In making these comparisons the obvious immaturity of the cysts and the fact that no living material was obtainable must not be ignored.

The cysts were somewhat irregular in shape but tended to be spherical in the larger individuals although the vulva usually remained visible. They varied from 0·35 by 0·23 mm. up to 0·56 by 0·38 mm. in size, with an average of 0·461 by 0·309 mm. The length of the neck varied from 0·08 mm. to 0·15 mm. The minimum ratio of length over thickness was 1·3, the maximum 1·6, and the average 1·48. The ratio of length over length of neck varied from 3 to 5·62, with an average of 3·83.

The eggs varied between 0·095 by 0·04 mm. and 0·12 by 0·05 mm. with an average size of 0·105 by 0·046 mm. The larvæ, as extracted from the eggs by gentle pressure measured from 0·28 mm. up to 0·413 mm. in length with an average length of 0·343 mm. and the average length of the stylet was 0·025 mm.

The adult males showed a considerable degree of variation, ranging from 0·8 mm. up to 1·84 mm. in length and with the ratio of length

over thickness varying from 25 to 41. The stylet measured from 0·02 mm. up to 0·025 mm. with an average length of 0·022 mm., and the spicules from 0·025 mm. up to 0·032 mm. with an average length of 0·028 mm.

The immaturity of the cysts renders their comparison with those of known strains of *H. schachtii* undesirable as misleading. The eggs of the nematode from maram grass were large, and corresponded almost exactly in size with those of the strain parasitic upon mangolds, but the larvæ were notably shorter than those of the mangold strain, although they corresponded with the latter in stylet-length. The adult males showed a greater variation in length than the males of any strain of *H. schachtii*, and the average lengths of stylet and spicules were shorter than any that have been recorded for this species.

In general structure the larvæ and males closely resembled those of *H. schachtii* occurring on cultivated crops, one difference in the structure of the males was however noted, viz., that in this nematode the post-anal region was considerably longer than in the adult males of *H. schachtii* previously studied by the present writer. In this particular, however, the species from maram grass agrees with the illustrations given by Cobb (1918), and Strubell (1888), of *H. schachtii*. The caudal papillæ were visible, and these also corresponded in position with those figured by Cobb.

Since the body-length and size of the spicules and stylet have been found to vary according to the host-plant, the abundance of the mucilaginous material and eggs outside the body and the constant presence of the male within the egg-mass are the only constant features by which this nematode appears to differ from *H. schachtii*. Whether this form should or should not be included within this species can only be determined by further observations carried out on living material.

The Possible Significance of Weeds as Hosts of *H. schachtii*.

Should this parasite of maram grass represent a true natural infection of *H. schachtii*, it seems possible that to it may be traced the source of some infections in cultivated areas. The reclamation of land around the sea coast must in many instances have involved the inclusion of large tracts which have borne sea maram grass.

The light sandy soil typical of reclaimed land, as, for example, large

areas of Lincolnshire, is especially suited to potato culture, and the common practice of repeatedly cropping such land with the one species would give to any parasite ideal conditions for adaptation to such a host. Following the lines of this suggestion, the fact that couch grass is parasitised more readily by certain strains of *H. schachtii* than are other species of weed more nearly related to the cultivated host, might be explicable as a tendency towards host-reversion on the part of the parasite.

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On some details of comparative anatomy in *Aphelenchus, Tylenchus* and *Heterodera.*

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INTRODUCTION.

In the microscopic examination of plant-parasitic and free-living nematodes it frequently happens that certain anatomical details stand out clearly whilst others remain obscure. Later examination of further examples of the same species, perhaps from a different source or at another season of the year, may reveal some of the previously obscure details with the result that structures which up to that time had been difficult to elucidate, are now seen distinctly in relation to their surroundings.

OPENINGS OF ŒSOPHAGEAL GLANDS.

Recent examination of specimens of *Aphelenchus avenæ*, obtained from decaying roots of Narcissus bulbs, proved very favourable for the detailed study of the stylet and œsophageal glands which had not been so clearly seen in specimens examined previously.

It may be noted that the name *œsophageal* rather than *salivary* is used intentionally for the reason that the glands are the homologues of the three occurring in the œsophagus of many well-known and widely different animal-parasitic nematodes in which they are generally referred to as œsophageal glands. Moreover, on general grounds, since their

function is not fully understood, it seems preferable to apply to them words which denote location rather than function.

The disposition of the œsophageal glands in *Aphelenchus* species relative to the muscular œsophageal bulb and the beginning of the intestine; is well known at the present time and has been dealt with in recent papers by Stewart (1921) and Goodey (1927 and 1928). In all species so far studied they lie outstretched as a more or less distinct band behind the bulb and dorsal or dorso-lateral to the first part of the intestine; appearing rather narrow and frequently with the posterior end rounded and visible under low magnification. The nuclei of the three cells composing the mass can often be seen under higher magnification but it is generally rather difficult to distinguish the limits of the cells themselves. Anteriorly, the blending of the glands with the immediately post-bulbar region of the alimentary canal is extremely difficult to differentiate particularly as just here the nerve-ring crosses the structures concerned.

Cobb (1923) in a note contributed to the Helminthological Society of Washington in October, 1922, called attention to the difference presented by *Aphelenchus* and *Tylenchus* species in regard to the opening of the dorsal œsophageal (salivary) gland. He showed that in both genera the two sub-ventral glands open into the lumen of the œsophagus at the base of the crescentic valves of the muscular bulb whereas the opening of the dorsal gland is differently placed in the two genera. In *Aphelenchus* it is situated within the muscular bulb in advance of the valves, appearing as a break in the cuticular lining of the lumen about halfway between the front of the bulb and the valves. In *Tylenchus*, on the other hand, the glandular material passes through the bulb, proceeds along the anterior part of the œsophagus dorsal to the lumen and opens into the latter by a short obliquely placed duct just behind the base of the stylet, vide fig. 2. This duct is generally comparatively easily seen in *Tylenchus* species even though the glandular material within the substance of the œsophagus may not be so easily found.

The writer's observations on the glands and their openings in *Aphelenchus avenæ* fully bear out Cobb's findings. The accompanying drawing (fig. 1) made under high magnification, shows the region of the alimentary canal concerned in lateral view and renders a lengthy

description unnecessary. The strand of granular material of the dorsal gland passing through the muscular bulb and its narrowing down to the point of opening into the lumen were well seen. The opening was seen as a break in the cuticular lining of the lumen a little in front of the

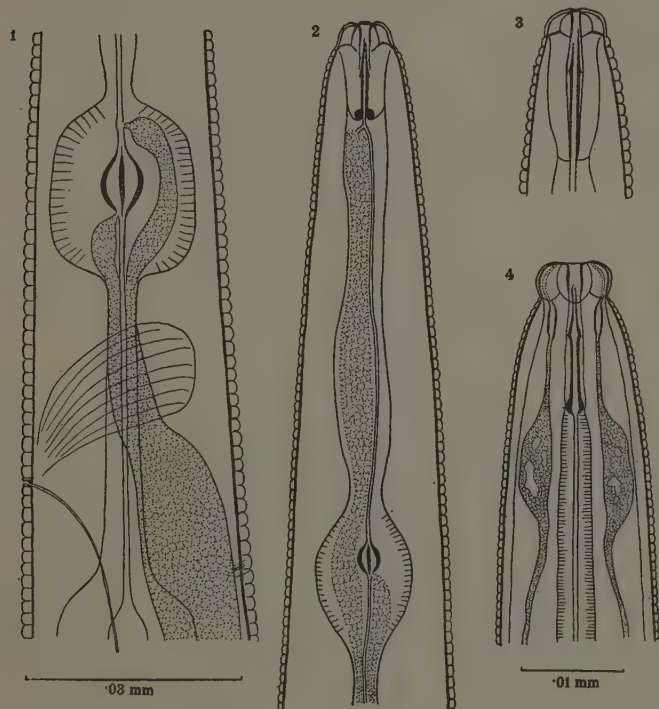

Fig. 1.—*Aphelenchus avenæ*—Lateral view of part of œsophagus showing opening of dorsal œsophageal gland in front of valves within muscular bulb and of a sub-ventral gland just behind valves.

Fig. 2.—*Tylenchus dipsaci*—Lateral view of anterior region showing opening of dorsal œsophageal gland by short duct behind base of stylet and of a sub-ventral gland just behind valves in bulb.

Fig. 3.—*Aphelenchus avenæ*.—Head end under high magnification showing shape of stylet.

Fig. 4.—*Aphelenchus parietinus*.—Anterior region in ventral view showing lateral ducts of cephalic glands.

crescentic valves. This break, as Cobb points out, is often quite easily visible owing to the refractive nature of the cuticular lining and when once seen is not difficult to find in other species of *Aphelenchus*. The openings of the sub-median glands, only one of which is shown in the figure, are found just where the valves return to the normal width of the lumen. The granular substance from the glands can frequently be seen to swell out here into two masses.

Since finding the above structures in *A. avenæ* the writer has re-examined numerous mounted specimens of different species of *Aphelenchus* and also some fresh material with the result that the break in the lumen of the bulb representing the opening of the dorsal œsophageal gland has been found in the following species: *A. fragariæ*, *A. ritzema-bosi*, *A. ribes*, *A. olesistus* from ferns and begonia, *A. pseudolesistus*, *A. parietinus*, *A. helophilus*, *A. demani*, *A. tenuicaudatus* and *A. winchesi*. It can therefore be reckoned as a constant feature characteristic of the genus.

Shape of the Stylet.

The same specimens of *A. avenæ* from Narcissus roots also showed that the stylet is not really plain throughout its length as previously figured by the writer and earlier workers, but is made up of two parts, an anterior conical portion, representing about one third its total length, united behind to a posterior cylindrical portion as shown in the accompanying fig. 3. Examination, under high magnification, of mounted specimens of *A. winchesi* obtained since the writer's earlier accounts of this species (Goodey, 1927), shows that it also has faint indications of an anterior conical part to the stylet and is not plain throughout as previously described. The same holds good also for *A. tenuicaudatus* though the division into the two regions is extremely difficult to resolve in the three mounted specimens available; the anterior portion showing up as rather more refractive than the posterior part. These three species, therefore, in possessing a stylet made up of two parts come into line in this respect with most other members of the genus.

Comparison of *Aphelenchus*, *Tylenchus* and *Heterodera*.

In the note by Cobb, already referred to, he points out that the location of the opening of the dorsal œsophageal gland may serve as a generic

character; it becomes a matter of interest therefore to compare the three plant-parasitic genera of nematodes in regard to this anatomical feature. When this is done it is found that the two species of *Heterodera*, *H. schachtii* and *H. radicicola* resemble *Tylenchus* in having the opening as a short duct just behind the base of the stylet. This is shown in drawings given by Cobb (1918) pp. 26-29 of a larva and male of *H. schachtii* and a larva of *H. radicicola* in all of which the three œsophageal glands are clearly depicted, situated very much as in *Tylenchus pratensis* and *T. musicola*.

The following table has been drawn up setting out the chief points of resemblance and difference presented by the three genera, Aphelenchus, Tylenchus and Heterodera.

1. General Body Form.

Aphelenchus.—Adults of both sexes worm-like.

Tylenchus.—Adults of both sexes worm-like.

Heterodera.—Females swollen and sac-like, males worm-like.

2. Male Characters.

Aphelenchus.—Tail usually tapering; lateral caudal wings absent; spicules paired, closely applied; gubernaculum absent; caudal papillæ present.

Tylenchus.—Tail usually tapering; lateral caudal wings present; spicules paired, separate; gubernaculum present; in some species a single post-anal lateral papilla present on either side.

Heterodera.—Tail bluntly rounded; lateral caudal wings absent, but cuticle somewhat inflated and flanged in lateral region; spicules paired, separate; gubernaculum present; caudal papillæ present, but difficult to see.

3. Female Characters.

Aphelenchus.—Vulva behind middle of body; gonad single and anterior, post-valval uterine sac frequently but not invariably present.

Tylenchus.—Vulva at middle or behind middle of body; in former case gonads paired and opposed, in latter gonad single, anterior and with post-vulval uterine sac.

Heterodera.—Vulva terminal, gonads paired.

4. Œsophageal Glands.

Aphelenchus.—Three nucleate glands present, usually located dorsal to the beginning of the intestine; opening of dorsal gland within muscular bulb in front of crescentic valves appearing as a break in the lumen, sub-ventral glands opening just behind valves.

Tylenchus.—Three nucleate glands present most frequently in the form of a spatulate or pyriform second swelling of the œsophagus traversed throughout by the lumen, but sometines, as in *T. pratensis*, *T. musicola* and *T. similis*, as an elongated glandular mass ventral or dorsal to the beginning of the intestine; opening of dorsal gland by a short oblique duct just behind base of stylet, opening of sub-ventrals as in *Aphelenchus.*

Heterodera.—Three nucleate glands present, best seen in larvae and males, difficult to see in adult female, lying as a glandular mass just ventral to the beginning of the intestine; opening of dorsal and sub-ventrals as in *Tylenchus.*

5. Stylet.

Aphelenchus.—In two parts, anterior conical joined to posterior cylindrical; base simple or with three swellings.

Tylenchus.—In two parts as in *Aphelenchus*; base always with three swellings.

Heterodera.—In two parts as in above; base with three large swellings.

Cephalic Glands (amphids) in *Aphelenchus parietinus.*

Just as the so-called salivary glands are homologues of the œsophageal glands of animal-parasitic nematodes so the glandular parts of the paired lateral organs or amphids may be homologised with the cephalic glands of these larger nematodes.

A female of *Aphelenchus parietinus* measuring 0·8 mm. long, obtained from some mint rhizomes was found, on being killed over a small flame, to be lying in ventral aspect. On examining the anterior end under oil-immersion a pair of lateral organs (amphids) was plainly visible, especially those parts of the structures lying on either side of the stylet. Fig. 4 shows their appearance and relative size well enough to render a detailed description unnecessary. Each consists of a narrow duct and

is made up of two parts ; an anterior portion with rather thick refractive walls united behind to the straight posterior portion lying parallel to the cylindrical part of the stylet. Each anterior part curved outwards slightly at the level of the posterior limit of the head. The greatest difficulty was experienced in attempting to determine the course of the ducts forwards. At first sight each appeared to open laterally in the groove which constricts the head behind, but on further examination the impression was obtained that the ducts passed through the substance of the head to openings on its anterior face as indicated by the dotted lines in the drawing. Such a position for the apertures corresponds with that described and figured by Steiner (1925) for the amphids of *Heterodera schachtii*, *Tylenchus dipsaci* and *T. tritici*.

The writer could find nothing within the ducts having the appearance of nerve fibres or terminals similar to those figured by Steiner in his Figs. 7 and 8. The posterior part of each duct was continued backwards for a short distance as a distinct channel lying in the substance of the body lateral to the œsophagus, but in attempting to trace it distally it became lost.

A point of special interest concerning the probable nature of these ducts is that about half-an-hour after the worm was mounted each duct appeared to become swollen behind and filled with a finely granular fluid in which certain clearer vacuoles or alveoli could be discerned. The impression obtained was, in fact, of some rather viscid liquid which had caused each duct to swell up in this region and that the ducts lead from some kind of secretory gland.

Such a view is in accord with that expressed by Cobb (1917), p. 128, who, under " Functions of the Amphids," says that he has found evidence of the outflow of a string of ribbon of coagulable substance from the amphid ducts when nematodes are fixed in Flemming solution. He states that he has traced the structures connected with amphids inwards and backwards and finds they have the appearance of ducts rather than nerve organs. The swelling of the duct described above is in accord with such a view of their function. It is, of course, quite probable that sensory nerves connected with lateral head papillæ occur alongside the ducts also ; Cobb, in fact, speaks of the presence of an innervated papilla in *Mononchus* and other genera very close to the amphids and suggests that different observers have confused two structures.

If the ducts described lead from glands what are the latter? The writer would suggest that they should be regarded as the cephalic glands. Such glands have been described by Looss (1905), pp. 54-55, in *Ancylostoma duodenale*, in which he says " they run up in the sides of the mouth capsule, and open through a minute slit at the base of the outer ventral tooth to the surface of the body; the aperture thus corresponds to the lateral head papilla."

Cephalic glands are also known in *Œsophagostumum dentatum* in which also the apertures are situated at the lateral head papillæ. In these two animal parasites the glands are continued backwards in the body, each as a large cell provided with a nucleus and with its protoplasm differentiated into an outer firm cortical layer and an inner softer axial mass which flows up and down when the animal moves. The homologising of these structures cannot be carried so far as to show that in *Aphelenchus* each gland is composed of a single nucleated cell, but at the same time a case can be made out for considering the ducts as leading from a pair of glands and, since the cephalic glands of animal-parasitic nematodes occupy the same relative position in the body, one may not unreasonably suggest that we are here dealing with the ducts of cephalic glands also. In other words we have uniformity of structure amongst the nematodes and not organs peculiar to that miscellaneous assemblage of worms loosely termed eelworms as distinct from the animal-parasitic forms.

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A New Record of the occurrence of a Tapeworm of the genus *Bertiella* in Man.

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DURING a visit to the island of St. Kitts, British West Indies, in 1928, I received from Dr. S. B. Jones for identification, a tapeworm which he had received from a young negress who had never left the island. The tapeworm was a specimen of *Bertiella*.

The island is over-run by West African green monkeys—there are no South American monkeys in the Lesser Antilles—and search was made among these for the reservoir host. In one, young specimens of *Bertiella* were found.

Baer has recently in his monograph of the Anoplocephalidæ, united all the old world simian species of *Bertiella* in the species, *B. studeri*, leaving the South American forms in the species, *B. mucronata*. The only differences of importance, however, between the two species appear to be the size of the egg and the distribution of the host. His account of the old world species is as follows :—

BERTIELLA STUDERI. (*Syn.* B. SATYRI, etc.).

The length is very variable—up to 800 mm., generally 2·5 to 3 cm. by 7—15 mm. long. The scolex is 0·7 to 1·3 mm. in diameter with four suckers 0·2 to 0·4 mm. The long muscle fibres consist of two

layers of fibres disposed in small concentric bundles of about 25 to 30 fibres each. The dorso-ventral and transverse musculature is well developed; but there are considerable variations even in the same specimen. Of the four longitudinal excretory vessels, the two ventral are larger and are united transversely. The ventral vessel is situated ventral to the dorsal vessel on each side of the segment. The sexual ducts pass dorsal to the excretory vessels and the nerves. The genital pore is in the anterior half of the lateral border of the segment. There are 150 to 300 testicles (each 60μ to 100μ in diameter), in one or two layers, extending through the entire breadth of the segment. The deferent canal is almost straight before opening into the cirrus pouch which is ·4—·6 mm. long and reaches almost to the ventral vessel on its own side. It is feebly muscular and contains an internal seminal vesicle and an unarmed cirrus. The vagina opens behind and ventral to the cirrus pouch and is surrounded by a layer of glandular cells of considerable variation which secrete a mucoid substance. The lumen of the vagina is muscular. There is a fairly large seminal receptacle. The ovary is poral, compact and strongly lobed. It is about ·2 to ·6 mm. wide. The yolk gland is small, kidney shaped and lies ventral to the dorsal face of the ovary and slightly poral to it. The uterus is a transverse tube limited by the excretory vessels which when gravid fills the entire segment. The eggs are 45μ to 60μ in diameter, and the embryos 10μ to 16μ surrounded by a well developed pyriform apparatus terminated by long filaments.

B. mucronata, according to Baer, is 150 mm. long by 10 mm. wide. There are about 140 testes in two or three dorso-ventral layers disposed in two horizontal rows—a single row being found behind the ovary. Each testes is 88μ to 100μ in diameter.

Occurrence in Man.

Cram has more recently described from man and imported chimpanzees in the West Indies, specimens which, after comparison with co-types, she identifies with *B. mucronata*. The maximum length of her specimens was 4 cm., there were about 100 testes and the ova measured 36μ to 40μ in diameter.

Meggitt has recently examined what appear to be co-types of *B. mucronata* from *Myceter niger* and *B. cercopitheci* from *Cercopithecus sp.*

from Looss' Egyptian collection. He concludes that there is no difference between these two specimens. As Baer has compared the latter species and *B. studeri* and found them identical, it seems probable that *B. mucronata* is identical with *B. studeri*.

Meggitt, however, describes from the Egyptian collection a new species, *B. fallax* from *Cebus capuchinus* as follows :—

The length is 175 mm. by 5 mm. wide with a scolex 0·5 mm. in diameter. The genital pores irregularly alternate, with a tendency to be unilateral, and lie in the anterior half of the proglottis margin. The genital cloaca has an external narrow portion followed by an inner globular part. The cirrus sac—·26 mm. long—extends to or just past, the excretory vessel. The vas deferens is only slightly coiled. There are about 200 testes in two groups, separated from the female glands but joined by one or more rows along the posterior margin of the proglottis ; the poral group is entirely posterior to the genital ducts, but the aporal extends to the anterior margin. The vagina is dilated as far as the poral edges of the ovary, then suddenly contracts to a thin tube. The ovary is median, crescentic with numerous outwardly directed finger-like lobes. The yolk gland is posterior to the ovary. The eggs are without a pyriform apparatus.

The locality of this species is given as " Egypt," but as *Cebus capuchinus* is a South American monkey, it is probable that the animal came from the Zoological Gardens in Cairo.

Dr. Jones' specimen from man from St. Kitts agrees with Cram's description except that it is about 7 cm. long, and is referred to *B. studeri*.

The specimens collected by me from *Cercopithecus sabaeus* are badly preserved as the animal had been dead for about eighteen hours before it reached me and the high temperature (90° F.) had caused a certain amount of decomposition. The largest specimen is 35 mm. long and 6 mm. broad. The morphology of the segments corresponds with Meggitt's description of *B. fallax*, but as there are no gravid segments present and the state of preservation is poor, a definite specific diagnosis is impossible.

It is probable that *B. studeri* occurs in the monkeys in St. Kitts—otherwise the specimen from the negress cannot be accounted for. The examination of better preserved material may show that this is the only specimen present.

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On *Hymenolepis sinensis* n. sp.; a New Cestode from the Grey Sand-Hamster (*Cricetulus griseus*).

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Introduction.

The material upon which this study is based was obtained from a Grey Sand-Hamster (*Cricetulus griseus*) which died in captivity at the experimental farm of the Institute of Agricultural Parasitology. Several examples of two species of hamster were received from Dr. Hindle on his return from China, the specimen furnishing the worms being one of these. On death a post-mortem examination of the animal was made and the cestodes removed and preserved in dilute formalin. Some weeks subsequently the parasites were examined and found to differ from the descriptions of other species of *Hymenolepis* encountered in hamsters and the present study was entered upon.

Technique.

The specimens which had been preserved for some time in 5 per cent. formalin were washed to rid them of the preservative and then stained in Ehrlich's acid hæmatoxylin at a strength of 1 part of stain to 10 parts of distilled water. Differentiation was accomplished in 70 per cent. alcohol 98 parts, HCl 2 parts, and subsequent blueing

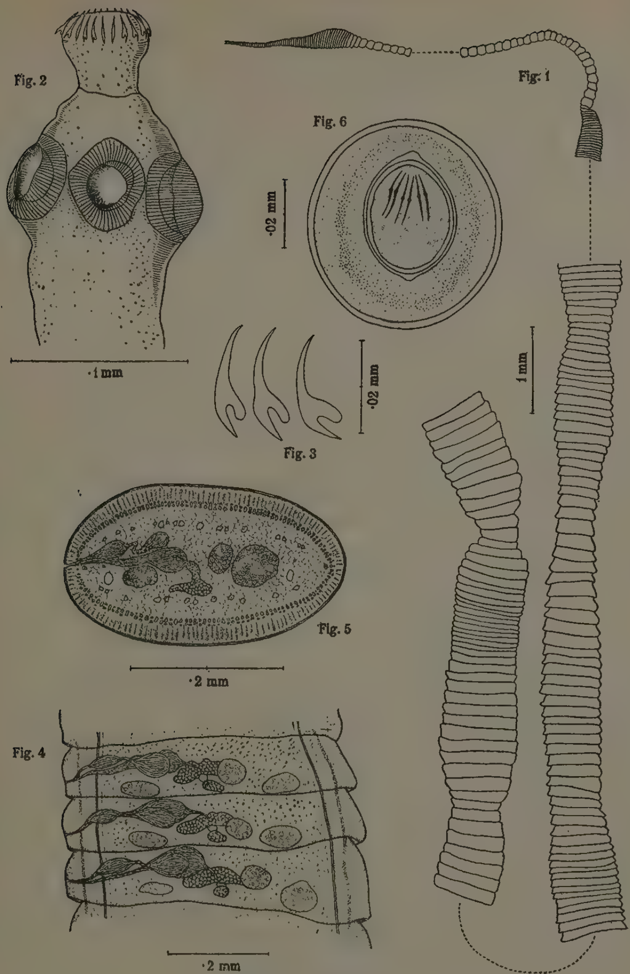
carried out by leaving the specimens in tap-water until sufficiently dark in colour when they were cleared in terpineol or creosote and mounted in balsam. This procedure gave excellent results for "in toto" mounts.

In order to procure pieces of the strobila for sectioning a preliminary light staining of an entire specimen was carried out with borax carmine so as to select a suitable part. This procedure allowed of several segments to be removed in a piece showing the sexual organs at the stage at which they were wanted for examination in sections. Several pieces were then carefully embedded and sectioned; the sections being easily recognised by their light red colour. After the requisite number of sections had been obtained they were stained by Clifford Dobell's modification of Mann's methyl blue, eosin stain (vide Bolles Lee's *The Microtomist's Vade-mecum*, 1921, p. 544).

The examination of the scolex was carried out by mounting it in lacto-phenol in which medium the hooks were clearly defined. The eggs were examined and drawn after being mounted temporarily in a mixture of equal parts of 5 per cent. formalin and normal saline.

Morphology.

The worm is very slender and semi-transparent or whitish. Complete specimens attain a length of 100 mm.; in a stretched condition they may measure 125 mm. According to the state of contraction the general form of the strobila may vary greatly and fig. 1 delineates a strobila which exhibits in different regions the state of contraction which can be found throughout an entire specimen. It follows that the external aspect of this species, as with several other cestodes, is of very doubtful systematic value unless the state of preservation and contraction is indicated. In specimens which exhibit little or no indications of contraction the maximum width of the strobila, which usually occurs in the region of the gravid segments, is from 0·54 to 0·88 mm.; exceptionally it may even reach 1·06 mm. Throughout, normal segments are always broader than long while in cases where contraction has markedly occurred in an antero-posterior direction the width may be as much as fifteen times the length.



Hymenolepis sinensis n. sp.

Fig. 1.—Parts of an entire strobila showing various states of contraction.
Fig. 2.—Scolex in dorsal view.
Fig. 3.—Hooks from scolex.
Fig. 4.—Three mature segments; dorsal view.
Fig. 5.—Transverse section through mature segment,
Fig. 6.—Egg.

The scolex (fig. 2), considerably wider than the " neck " which follows it, is more or less rectangular in transverse section and somewhat flattened dorso-ventrally although the shape may vary depending on the state of contraction. The scolex measures at its widest point across the region of the suckers from 0·08 to 0·12 mm. Each of the four suckers, which are situated laterally, have an outside diameter of 0·043 to 0·051 mm. They are more or less round, large, not particularly deep, and usually touch or almost touch one another. The scolex bears a well-developed rostellum, 0·046 to 0·048 mm. in diameter, armed with a single row of hooks of the characteristic shape. There are 20 hooks present measuring 0·022 to 0·024 mm. in length (fig. 3).

The " neck," unsegmented for a short distance behind the scolex, reaches a length of 2 mm. when signs of segmentation become visible. The first traces of genital organs in the form of very small, centrally situated, deeply staining masses begin to be recognisable at about the 40th to 45th segment from the anterior end. At about the 300th segment fully developed male and female organs can be seen. Gravid segments vary in number from approximately 150 to 250, while the total number of recognisable segments in the strobila may reach 2,000 or more.

In a mature segment (fig. 4) the male sexual apparatus consists of three testes, round to ellipsoidal in shape. Their greater diameter measures 0·05 to 0·08 mm. and their smaller 0·03 to 0·06 mm. Two of the testes are located in the posterior border of the proglottid, the third being anterior and median to the posterior antiporal testis. In those segments which show marked contraction the position of the third testis is such that it may almost fall into the transverse line formed by the two posterior testes but in the great majority of the proglottides it is more or less anterior and median to the antiporal testis. In no case has it been observed to become directly anterior or lateral to the posterior antiporal testis although it has approached near to the first position.

In their description of *Hymenolepis microstoma* (Dujardin, 1845) Joyeux and Kobozieff (1928) point out that that species, in which a great latitude of variation in the position of the testes is encountered, is referable generally to the genus *Wienlandia* as proposed by Mayhew (1925). In segments which have contracted, however, the diagnostic

characters of the genus *Wardium* Mayhew, 1925, are met with, the position of the testes being variable. In the species under discussion the characters of the genus *Wienlandia* Mayhew, 1925, apply in the majority of cases although at times the position of the testes almost merit its inclusion in the genus *Hymenolepis*, *sensu stricto*, of Mayhew. The writer agrees, under these circumstances, with the remarks made by Joyeux and Kobozieff that it is difficult to appreciate the characters proposed by Mayhew, especially in material indifferently or badly fixed and exhibiting much contraction ; also, Mayhew only dealt with the Hymenolepididæ of birds although it is to be supposed that the classification he proposes ought to apply to all species even though the host be mammalian. With reference to Mayhew's classification, Baylis (1929) makes the following note : " . . . It appears doubtful whether, in different states of contraction, the testes even of a single individual maintain a sufficiently constant position for such a classification to have any definite value. In any case, groups based primarily upon this character should probably not be given higher rank than that of subgenera of *Hymenolepis*."

The genital pore occurs without exception on the left side of the strobila, when viewed dorsally, and is situated a short distance posterior to the mid point of the lateral border of the segment. The cirrus-sac (fig. 5), measuring about 0·1 mm. in length, passes dorsally to both dorsal and ventral excretory vessels and to the longitudinal nerve. It assumes the usual club-shape encountered in other species dilating towards its inner end and attaining a width of 0·02 to 0·03 mm. It is almost completely filled by the internal seminal vesicle. Leading from the cirrus-sac is a narrow canal, at times almost straight at other times very sinuous, which dilates and forms the external seminal vesicle. It is also pear-shaped and practically occupies the space between the ovary and the inner end of the cirrus-sac. The large receptaculum seminis lies ventrally to the external seminal vesicle and is formed from the expanded inner end of the vagina which, extending along the ventral side of the cirrus-sac, dilates gradually as it leaves the genital pore.

The ovary is a fairly conspicuous organ, compact and not very markedly lobed, except on occasion and then irregularly, and occupying roughly one-quarter of the width of the segment. Its side to side measurement is from 0·13 to 0·14 mm. The vitelline gland is spherical

or ovoid, situated posterior and dorsal to the ovary near the median line. The shell-gland is smaller, globular and lies beneath the yolk-gland.

The female sexual organs originate, as already mentioned, as small cellular masses which increase very gradually in size while the male sexual organs undergo a more rapid development. Male and female organs are found in full maturity in about the 300th segment. From this point onwards the male and female organs commence to degenerate and slowly disappear, the latter giving place to the uterus so that the passage from mature to gravid segments is very gradual. In gravid segments the sac-like uterus almost completely fills each proglottid and contains a large number of ova.

The ovum (fig. 6), nearly spherical, is composed of a thin outer membrane and an inner shell which is thicker and more chitinised. In the space between the two shells is a quantity of granular material. The inner shell is more or less lemon-shaped and possesses a well-marked thickening at each pole; the terminal filaments encountered in the eggs of other species of *Hymenolepis* appear to be absent. Eggs from gravid segments gave the following measurements: outer membrane, 0·076 to 0·052 mm. × 0·064 to 0·048 mm., average 0·061 × 0·054 mm.; inner shell, 0·042 to 0·029 mm. × 0·029 to 0·025 mm., average 0·036 × 0·027 mm.; knobs at poles, 0·002 to 0·004 mm.; onchosphere, 0·032 to 0·028 mm. × 0·025 to 0·022 mm., average 0·030 × 0·024 mm.; length of embryonic hooks, which are of the usual shape, 0·014 to 0·016 mm.

Discussion.

Two species of *Hymenolepis*, parasitic in the hamster, have already been found and described. These are *Hymenolepis straminea* (Goeze, 1782) and *H. criceti* Janicki, 1904. As much information as possible concerning these two forms has been collected and a thorough comparison made with the species under examination. As a result the writer has been unable to convince himself that he is dealing with either *H. straminea* or *H. criceti* and proposes, in consequence, to regard the

species now studied as new and to give to it the name of *Hymenolepis sinensis* n. sp. as indicative of the country of origin rather than attempt to show from what host it was taken as such a procedure might result in confusion with the already existing *H. criceti* Janicki, 1904.

Joyeux and Kobozieff (1928) remark on the fact that, amongst the *Hymenolepis* from rodents, there are a certain number of species which have been described by authors whose works are now very old so that identification is difficult. Into this group of ill-defined species falls *H. straminea* (Goeze, 1782). On the other hand with *H. criceti* described comparatively recently much more detailed information is available. In 1904 Janicki created this species and gave a brief description of it but later (Janicki 1906) augmented the account by a much fuller diagnosis. A tabulated comparison of the three species involved in this paper has been added but it seems opportune to indicate here the main points of difference between *H. criceti* and *H. sinensis*. The former cestode, from *Cricetus cricetus*, has an appreciably wider scolex, with 24 hooks, than that of the latter which only bears 20 hooks. Janicki's figure (1906, Pl. xxii., fig. 38) of an individual hook shows it to be quite different in shape and length (0·016 mm.) from that of *H. sinensis* (0·022 to 0·024 mm.). With regard to the egg the longer diameter of the outer membrane is 0·043 mm., of the onchosphere 0·025 mm., in *H. criceti*; in *H. sinensis* the corresponding average measurements are 0·061 mm. and 0·030 mm. One further point, but one upon which too much stress cannot be laid owing to the varying states of contraction of proglottides when killing and fixing occurs, is the ratio of length to breadth in the segments. Janicki states that in fully developed segments the length to breadth is as 1 to 1·5 gravid segments even becoming square. In those segments of *H. sinensis* which appear normal exhibiting little or no contraction the length to breadth is approximately as 1 is to 3 or 4.

Janicki (loc. cit.) took his specimen from glass No. 2038, which was labelled "*Tænia straminea*," of the Berlin Zoological Museum and points out that there is not the slightest doubt that his new species is to be separated from *H. straminea* of Goeze by having relatively long segments, the want of a projecting posterior border, and small breadth of the strobila. While these characters are hardly sufficient in themselves an examination of all the available characters of *H. straminea* and a

comparison with those of *H. criceti* convinces one that the two species are quite separate and distinct.

In 1782 Goeze described from a hamster a cestode which he called *Tænia straminea* owing to its similarity to a "blade of straw." There is little useful information in his description except that he noted the scolex bore four suckers and very fine hooks, that it measured up to almost 8 inches long and appeared as a very narrow fragile strobila, and that the eggs, like "shuttles," contained distinct embryos. His figures, unfortunately, are of little help. Other writers such as Batsch (1786), Blanchard (1891), Bosc (1827), Bruguière (1791), Cobbold (1879), Diesing (1850), Gmelin (1790), von Linstow (1878), Rudolphi (1809, 1810, 1819), Schrank (1792), and Zeder (1803) have summarised and occasionally supplemented the original description by Goeze. Dujardin (1845) who also summarises to some extent Goeze's description states that the length of the strobila is 30 to 200 mm. with a breadth of 1·2 to 2·25 mm. *H. sinensis* measures about 100 mm. or even 125 mm. in a fully stretched condition with a breadth of from 0·54 to 0·88 mm. and exceptionally up to 1·06 mm.

Kowalewski (1894 and 1895), however, adds the useful information that the scolex bears 19 to 23 hooks, 0·014 mm. in length. A figure of two hooks (1895, Pl. viii., fig. 28) is given and their shape differs considerably from that of *H. sinensis*.

It was not until 1913 that a more detailed account became available when Cholodkovsky gave a short redescription of the cestode and placed it into the genus *Hymenolepis* although Blanchard (1891) recognised years before that it belonged to that genus. From Cholodkovsky's redescription we find that the length of the hooks on the scolex is 0·014 mm., exactly the same measurement as given by Kowalewski (vide supra), but no information as to their number is mentioned. The width of the scolex of *H. straminea* is given as 0·2 mm.; in *H. sinensis* it is only 0·08 to 0·12 mm. The eggs of *H. straminea* are noted as being oval with a longer diameter of the outer membrane of 0·063 mm. In *H. sinensis* the ovum is almost spherical and the corresponding average measurement is 0·061 mm. The description of the disposition of the testes does not agree with the figure (fig. III) given by Cholodkovsky and attention to this discrepancy has been pointed out by Joyeux and Kobozieff (loc. cit.) who say: "Cholodkovsky dit que les testicules

sont situés en une rangée transversale, mais sa figure (fig. 3) montre les deux antiporaux en ligne transversale, ou oblique, ou l'un derrière l'autre, suivant les anneaux." Whether the statement or figure is taken as definite it must be noted that in *H. sinensis* the two antiporal testes have never been found to be in a transverse line or the one behind the other although they have occasionally approached these two situations. It is to be regretted that Cholodkovsky's figures are small and lack detail and in the case of the hook from the scolex it would appear that only the blade and part of the ventral root have been figured which makes a comparison of shapes almost impossible.

Further, directing attention to the hosts of the cestodes under discussion *H. sinensis* parasitises *Cricetulus griseus* A. Milne-Edwards, the Grey Sand-Hamster, whose habitat is given in the Zoological Society of London's *List* (1929) as: "North-Eastern Asia; North China and Mongolia." *H. straminea* and *H. criceti* both occur in *Cricetus cricetus* Linnæus (syn. *C. frumentarius*) the habitat of which is given as: "Europe: from Belgium and Northern France eastward to Russia and Asia Minor." *Cricetus vulgaris* has been cited as synonymous with *C. cricetus*, the Common Hamster, and although this name does not appear in the above-named *List*, it would appear to be in order. Curiously enough Dujardin (1845) mentions the name of *Arctomys cricetus* for the hamster but it is probable that an error has been made in the generic name.

In conclusion it can be said that, while the morphological differences between *H. criceti* and *H. sinensis* are fairly evident, those between the latter cestode and *H. straminea* are less well marked due mainly to the fact that accurate information about *H. straminea* is somewhat scanty despite the number of references which have been consulted. If, however, we consider in addition the geographical distribution of the hosts of these two worms there seems that added support and justification is given to the consideration that the species described in this paper is a new one.

The following table of measurements and other data will help to demonstrate some of the differences between the three species which have been under discussion.

—	*Hymenolepis sinensis* n. sp.	*Hymenolepis criceti* Janicki, 1904.	*Hymenolepis straminea* (Goeze, 1782).
Host from which described	*Cricetulus griseus*	*Cricetus cricetus C. vulgaris*	*Cricetus cricetus C. vulgaris*; *C. frumentarius* Pall.; *Arctomys cricetus*
Location	Small intestine	Intestine	Small and large intestine.
Length	Up to 100 mm.; 125 mm. if stretched		30-200 mm.
Width of strobila	0·54 to 0·88 mm.; 1·06 mm. in very contracted specimens	0·44 mm.	1·2 to 2·25 mm.
Width of scolex	0·08 to 0·12 mm.	0·314 mm.	0·2 mm.
Suckers, outside diameter	0·043 to 0·051 mm.		
Rostellum, diameter	0·046 to 0·048 mm.		
Number of hooks	20	about 24	19 to 23
Length of hooks	0·022 to 0·024 mm.	0·016 mm.	0·014 mm.
Number of segments	2,000 or more		
Segment at which rudiments of genital organs begin to appear (roughly)	40-45th		
Segment at which mature genital organs appear (roughly)	300th		
Number of gravid segments (roughly)	150 to 250		
Ova, diameter of outer membrane	0·076 to 0·052 mm. × 0·064 to 0·048 mm.	0·043 × ? mm.	0·063 × ? mm. (oval)
Ova, diameter of inner shell	0·042 to 0·029 mm. × 0·029 to 0·025 mm.		
Ova, diameter of onchosphere	0·032 to 0·028 mm. × 0·025 to 0·022 mm.	0·025 × ? mm.	
Embryonic hooks	0·014 to 0·016 mm.		
Breadth : length of segments	Always broader than long, 3–4 : 1; 15 : 1 on occasion	1·5 : 1, last segments square	4 : 1

SPECIFIC DIAGNOSIS OF *Hymenolepis sinensis* n. sp.

Adult strobila up to 100 mm. long, 125 mm. when fully stretched, semi-transparent or whitish throughout. Scolex small, more or less rectangular in transverse section, 0·08 to 0·12 mm. wide. Four suckers, situated laterally, with an outside diameter of 0·043 to 0·051 mm., more or less round and touching or almost touching one another. Rostellum well developed, 0·046 to 0·048 mm. in diameter, armed with a single row of 20 hooks measuring 0·022 to 0·024 mm. in length. Neck long and slender becoming segmented at 2 mm. from scolex. Genital organs first visible in 40th to 45th segment. Proglottides with fully functional male and female organs at about the 300th segment. Gravid segments number from 150 to 250, measuring from 0·54 to 0·88 mm., exceptionally up to 1·06 mm. Total number of recognisable segments 2,000 or more. Genital pore slightly posterior to mid point of lateral border of segment. Testes measure 0·05 to 0·08 × 0·03 to 0·06 mm. arranged as follows: one posterior and poral, one posterior and antiporal, the third anterior and median to the posterior antiporal testis. Cirrus-sac measures 0·1 mm. in length and passes dorsally to dorsal and ventral vessels and longitudinal nerve. Ovary compact and not markedly lobed measuring 0·13 to 0·14 mm. from side to side. Egg nearly spherical with average measurements as follows: outer membrane, 0·061 × 0·054 mm.; inner shell, 0·036 × 0·027 mm.; knobs at poles, 0·002 to 0·004 mm.; onchosphere, 0·030 × 0·024 mm.; embryonic hooks 0·014 to 0·016 mm. long.

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On the Trematodes of the digestive tract of *Tropidonotus piscator* from Lucknow.

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Reptiles form a very prominent part of the fauna of India, but very little is known about the Entozoa of this group in the country. The foundation of our knowledge of the Trematode parasites of Reptiles is laid by Rudolphi (1819), Dujardin (1845) and Diesing (1850-58), all of whom have described them under a common genus *Distomum*, along with the forms from other hosts. Von Linstow (1877-85), Looss (1899) and Lühe (1900-11) have considerably extended our knowledge of these forms. They have also revised the observations of the earlier workers and have split up the original genus into several genera, thereby placing the group on a more scientific and natural basis.

Amongst the recent investigators on the Reptilian trematodes, the name of Nicoll stands prominent. This able investigator has worked out the entozoa of reptiles that died at the Zoological Gardens, London, and has described several new forms. He (1911) described a new Trematode from *Tropidonotus rhombifer*, and later (1912) gave an account of two larval trematodes from *Tropidonotus ordinatus*. In 1914, Nicoll described several reptilian trematodes, including two new species from *Tropidonotus ordinatus* and *Tropidonotus piscator* respectively. In the same communication, he gives an account of a new genus *Ommatobrephus* from the intestine of *Uromastix acanthinurus*. Recently Bhalerao (1926) described some forms from the intestine of *Tropidonotus piscator* in Burma.

In the course of our investigations on the helminth parasites of *Tropidonotus piscator* at Lucknow we obtained a few specimens of trematodes belonging to two different genera, one of which, on closer examination, appears to be new species belonging to the genus *Ommatobrephus* Nicoll, 1914. It would be worth while to mention here that while *Ommatobrephus singularis* was obtained from *Uromastix*, a terrestrial host, the present species is obtained from the water snake. All the specimens were attached to the intestinal wall of the host by their large ventral sucker, but are easily detached from it. Their anatomy is easily visible by a slight pressure of the coverslip.

I. Ommatobrephus folium n.sp.

This fluke is thin leaf-like and is a small distome 2·5—3·15 mm. in length. Its characteristic feature is the presence of a large number of eggs in the uterus. It has its greatest breadth of 1·46 mm. behind the ventral sucker. The body shows a considerable degree of contractility and tapers anteriorly, while the posterior end is broad and rounded. The general surface of the body is smooth and does not bear any spines.

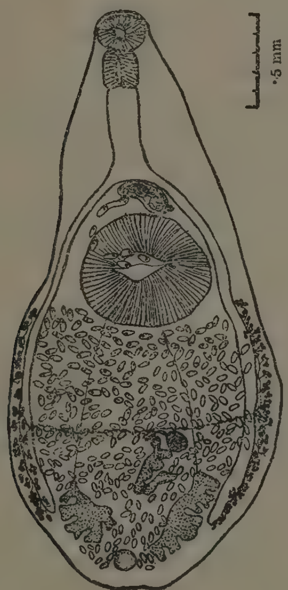
The oral sucker is smaller than the ventral sucker and both are transversely elongated, the ratio between the oral and the ventral sucker is 1 : 3. The oral sucker is situated at the anterior end of the body and measures 0·2 mm. in length and 0·26 mm. in breadth.

The genital pore is situated in front of the ventral sucker between it and the crura of the intestine, being nearer the former. Its distance from the ventral sucker is 0·07 mm. The excretory pore is sub-terminal and the excretory vesicle is Y-shaped. It exhibits, together with its branches, a characteristic feature of the family Lepodermatidæ.

Immediately following the oral sucker is a large elongated pharynx with well developed musculature. It is 0·2 mm. long and 0·18 mm. broad. There is no prepharynx. The œsophagus is more than twice the length of the pharynx, fairly broad, and is 0·42 mm. long and 0·15 mm. broad. It bifurcates into two narrower cœca which extend to the posterior end of the body as far back as the testes.

The testes are connubial and are situated at the posterior end of the body, separated from each other by the coils of the uterus. They are

unequal in size, the left one being larger than the right. They are flat, irregularly lobed structures situated completely within the cœca. The irregular lobulations of the testes are peculiar to the species. The vasa deferentia emerge from the anterior end of the testes, but are obliterated owing to the enormous development of the eggs within the uterus. The



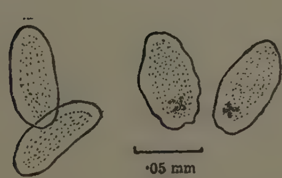


Fig. 1.—*Ommatobrephus folium*, ventral view.
Fig. 2.—Eggs and Miracidia within the egg shell.

cirrus sac is situated in front of the ventral sucker in the space between it and the bifurcation of the intestine. It is oval in shape and measures 0·27 mm. in length and 0·13 mm. in breadth, having thick muscular walls. Within it is enclosed a spherical vesicula seminalis, 0·12 mm. long. This latter leads into a short, narrow, slightly coiled ductus ejaculatorius surrounded towards its posterior end by a large number of pear-shaped unicellular prostate gland cells. The terminal part of this duct is muscular and forms a narrow cirrus.

The ovary is a small pear-shaped structure situated in front of the testes, but slightly towards the left. It is 0·15 mm. long by 0·1 mm. broad. Behind it is a large irregular receptaculum seminis, connected in front with a long sinuous Laurer's canal running above the receptaculum seminis. The general nature and disposition of the latter is of the same character as is found in the genera *Encyclometra* (=*Odhneria*) and *Tremiorchis* (=*Centrovitus*), there being only one opening for it and the receptacle. This point is marked by the presence of indistinct follicles of the shell gland.

The vitelline glands are composed of several distinct follicles arranged along the outer side of the intestinal crura and extend between the ventral sucker in front and the terminations of the intestine behind. The transverse vitelline ducts run behind the ovary and meet each other on the right side of the latter and then join the oötype.

The uterus is very voluminous and occupies the entire area between the crura behind the ventral sucker to the posterior end between the two testes. Both the ascending and descending limbs are so intimately intertwined that it is difficult to recognise them from each other. It extends forward on the ventral surface of the acetabulum and then curves to the right to join the male duct at the genital atrium. The eggs are operculated, oval and have a thin transparent shell. In the upper part of the ascending limb of the uterus the majority of the eggs contain miracidia, whose kidney-shaped eyespots are conspicuously visible through the thin egg-shell even under the low-power of the microscope, and thus resemble the genus *Encyclometra* (= *Odhneria*). The eggs measure 0·06 × 0·03 mm. in dimensions.

From the foregoing description, it would be evident that the present form differs from *Ommatobrephus singularis* Nicoll, 1914, in the following features :—

1. The intestinal crura extend right backward to the posterior end and do not stop midway between the acetabulum and the ovary.

2. Testes are deeply lobed, and are widely separated from each other by the uterus.

3. The vitellaria are lobed follicles and also extend further backward than in the species described by Nicoll.

These features appear to be sufficient to justify the creation of a new species for the present form.

Locality in the host.—Small intestine of *Tropidonotus piscator*, Lucknow.

II. Acanthochasmus burminis Bhalerao, 1926.

This form has been described by Bhalerao from Burmese snake, *Tropidonotus piscator*, and we obtained a large number of specimens of this form in Lucknow from the small intestine of the same host along with *Ommatobrephus folium*. It therefore records its occurrence at Lucknow also. There is nothing to add to the description given by Bhalerao (1926).

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CORRIGENDA.

Page 9, 14th line from bottom, for "*ate*" read "*Rate.*"